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The Manitoba Personalized Lifestyle Research (TMPLR) study protocol: a multi-centre bi-directional observational cohort study with administrative health record linkage investigating the interactions between lifestyle and health in Manitoba, Canada

Journal:	<i>BMJ Open</i>
Manuscript ID	bmjopen-2018-023318
Article Type:	Protocol
Date Submitted by the Author:	31-Mar-2018
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	University of Manitoba; University of Manitoba, Department of Food and Human Nutritional Sciences
Keywords:	EPIDEMIOLOGY, Health informatics < BIOTECHNOLOGY & BIOINFORMATICS, NUTRITION & DIETETICS, Physiology < BASIC SCIENCES

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The Manitoba Personalized Lifestyle Research (TMPLR) study protocol: a multi-centre bi-directional observational cohort study with administrative health record linkage investigating the interactions between lifestyle and health in Manitoba, Canada

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Keywords: nutrition, physical activity, sleep, chronic disease, genetics, microbiome

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Word Count:3741

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2
3 **80 Abstract**

4
5 **81 Introduction**

6
7 82 Lifestyle factors, such as diet, physical activity and sleep, are associated with the development of
8 83 many chronic diseases. The objective of The Manitoba Personalized Lifestyle Research
9 84 (TMPLR) study is to understand how these lifestyle factors interact with each other and
10 85 additional factors, such as an individual's genetics and gut microbiome, to influence health.

11
12 **86 Methods**

13 87 An observational study of adults, with deep phenotyping by objective health and lifestyle
14 88 assessments, multi-omic analyses, and retrospective assessment of early life experiences, with
15 89 retrospective and prospective utilization of secondary data from administrative health records.

16
17 **90 Study population**

18
19 91 A planned non-random convenience sample of 840 Manitobans aged 30-46 recruited from the
20 92 general population, stratified by sex (equal males and females), body mass index (BMI; 60% of
21 93 participants with a BMI >25), and geography (25% from rural areas, accessed using a mobile
22 94 research unit).

23
24 **95 Measurements**

25 96 Lifestyle factors assessed will include dietary pattern, physical activity, cardiovascular fitness
26 97 and sleep. Additional factors such as medical history, socio-economic status, alcohol and tobacco
27 98 consumption, cognition, stress and anxiety, and early life experiences will also be documented.
28 99 A maternal survey will be performed. Body composition and bone density will be measured by
29 100 dual energy x-ray absorptiometry. Blood pressure, pulse wave velocity, and augmentation index
30 101 will be measured on two consecutive days. Chronic disease risk biomarkers will be measured in
31 102 blood and urine samples. DNA will be extracted for genetic analysis. A fecal sample will be
32 103 collected for microbiome analysis. Participants may provide their Manitoba Personal Health
33 104 Information Number (PHIN) to link their study data with administrative health records.

34
35 **105 Ethics and dissemination**

36
37 106 Ethics approval has been obtained from the University of Manitoba Health Research Ethics
38 107 Board (protocol # HS18951; 05/01/2016). Data analysis, release of results, and publication of
39 108 manuscripts are scheduled to start in late 2018. Additional information at www.TMPLR.ca.

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41 **109 Clinicaltrials.gov NCT#xxxxxx**

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3 111 **Article Summary**

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5 112 **Strengths and limitations of this study**

6 113 The study is designed to capture deep phenotyping of participants in the areas of diet, physical
7 114 activity and sleep, combined with genetic and gut microbiome profiles. The ability to link these
8 115 study data to healthcare usage data retrospectively and prospectively is a key strength of the
9 116 research (**Figure 1**). The use of a mobile research unit to access rural populations makes the
10 117 study unique as geographic setting can strongly influence health-related behaviors.

11 118 The study uses non-random convenience sampling for feasibility reasons, which can introduce
12 119 selection bias and limit generalizability; however, preset stratification based on sex, BMI, and
13 120 geography have been implemented to enhance representation. Another limitation is that some of
14 121 the questionnaires used in TMPLR have not previously been validated, or not validated in the
15 122 specific TMPLR study population. Finally, the study sample size of 840 individuals was not
16 123 selected to power a specific primary hypothesis; however, it will provide a one-of-a-kind
17 124 research platform of deeply phenotyped participants in which to investigate the associations
18 125 between lifestyle and chronic disease.

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127 **Introduction**

128 Approximately half of Manitobans are living with at least one of the following chronic
129 conditions: obesity, hypertension, type 2 diabetes (T2D), cardiovascular disease (CVD), or
130 chronic kidney disease (CKD) [2]. The consequences of these chronic conditions is substantial
131 and the financial burden, both personally and societally, is enormous. In the province of
132 Manitoba, which has a universal healthcare system and a population of approximately 1.2
133 million, over 40 percent of total provincial revenues are spent on healthcare[1]. The burden of
134 conditions like T2D and CKD are not unique to Manitoba [2, 3], therefore the primary and
135 secondary prevention of these chronic conditions is a major international health research priority
136 [4] .

137 It is well established that diet, physical activity, and sleep influence health and mortality [5-8].
138 Evidence-based guidelines pertaining to nutrition, physical activity and sleep exist to educate the
139 public on healthy lifestyle choices. However, most current lifestyle guidelines follow a one-size-
140 fits-all format, even though they are intended for populations comprising individuals with
141 diverse and complex health circumstances and unique factors influencing their ability to follow
142 the guidelines. This format may be a contributing factor to the poor adherence to lifestyle
143 guidelines. For example, although most people are aware that physical activity is important for
144 health, only 15% of the Canadian population achieve the national recommendations [9].
145 Similarly, it is estimated that 50% of women and 70% of men in Canada have energy intakes that
146 exceed their energy needs, while 50% to 90% have deficiencies in calcium and vitamin D [10].

147 There is now an increasing interest in the creation of lifestyle strategies or guidelines for specific
148 sub-populations or groups of individuals with specific characteristics [11-13]. It is hoped that
149 such tailored recommendations will be more effective, and that barriers to healthy lifestyle
150 practices can be ameliorated through personalization. Current one-size-fits-all recommendations
151 and strategies may not be effective due to (1) significant inter-individual variability or (2) shared
152 circumstances, such as geography, sleep/wake pattern, or socio-economic status, of a particular
153 group.

154 We hypothesize that an individual's lifestyle will be influenced by socio-economic status and
155 geography, and will interact with their genotype and gut microbiota to affect health [14, 15].
156 Accordingly, The Manitoba Personalized Lifestyle Research (TMPLR) study will involve the
157 coordinated collection of data related to socio-economic status, geography, nutrition, physical
158 activity, sleep, early life experiences, and health systems usage, in conjunction with the analysis
159 of genetics, gut microbiota, and risk factors for chronic conditions such as obesity, hypertension,
160 T2D, CVD, and CKD. After establishing the baseline characteristics of this study cohort,
161 administrative health records will be used retrospectively to examine the developmental origins
162 of health and disease, and prospectively to track and investigate the development of chronic
163 disease in the future. Consent has been obtained to contact study participants for further clinical
164 assessments, contingent on future funding.

165 Data from this study will provide an ideal opportunity for the discovery of new interactive
166 mechanisms through which lifestyle factors affect health. These interactive mechanisms may
167 also address the question of why some people are more successful than others in changing their
168 lifestyle as it relates to chronic conditions. Findings from this research will be useful in guiding
169 both clinical and health policy decisions, and will also facilitate the design and testing of
170 personalized health promotion strategies.

171 **Methods**

172 **Design**

173 This is an observational cohort study with retrospective and prospective utilization of secondary
174 data from administrative health records (**Figure 1**). The Strengthening the Reporting of
175 Observational Studies in Epidemiology (STROBE) guidelines were followed where applicable in
176 the development of this protocol manuscript [16].

177 **Setting**

178 Urban (Winnipeg) and rural (Morden, Winkler, Carman, Steinbach) areas with road access in
179 southern Manitoba, Canada.

180 **Objectives of the study**

181 The objective of this study is to explore the complex interactions that exist between lifestyle,
182 genetics, and gut microbiota, and how these relate to risk factors for chronic conditions,
183 especially obesity, hypertension T2D, CVD, and CKD in Manitoba.

184 **Inclusion and exclusion criteria**

185 A sample of 800 Manitobans aged 30-46, stratified by sex, BMI, and geography (**Table 1**) are
186 being recruited. Participants must have lived in Manitoba for a minimum of 5 years. Women
187 who are pregnant or lactating are not eligible to participate. Additionally, because it is expected
188 that very few of the 800 Manitobans who join TMPLR study from the general public will have
189 reduced kidney function (eGFR <30 ml/min), 40 participants (20 female, 20 male, with no set
190 stratification based on BMI or geography) who have severely reduced kidney function are being
191 recruited from the renal health clinic at Seven Oaks General Hospital (SOGH), Winnipeg, MB.
192 Therefore, the study has a recruitment goal of 840 participants.

193 **Recruitment**

194 Participants are recruited through the use of printed flyers, online advertisements purchased via
195 Google, Facebook, and Twitter ad platforms and social media accounts, appearances in local TV,
196 radio, and print media, and direct contact with community groups, such as churches, sports
197 leagues, and community clubs. All patients who receive care in the SOGH renal health clinic,
198 who are aged 30-46, have been living in Manitoba for a minimum of the last 5 years, and are
199 able to provide informed consent are approached to enroll in the study as well.

200 **Sample size**

201 The sample size of TMPLR study was selected based on considerations of feasibility of
202 recruitment, costs, and logistics. However, given established values from other sources [17] and
203 our anticipated sample size of 840 participants, we estimate that we will have 80%
204 power (5% significance, 2-sided) to detect a minimum body fat difference of 2.5% for rare
205 exposures (i.e., experienced by 10% of participants, such as smoking) and 1.7% for more
206 common exposures (experienced by 25% of participants, such as meeting the Canadian
207 recommended 150 minutes of moderate-to-vigorous physical activity). Additional estimated
208 minimum detectable differences are presented in **Table 2**. These lower limits should allow for
209 the detection of clinically meaningful changes in these outcomes.

210

211 **Data Collection and Assessments**

212 On two consecutive days, participants come to either the urban TMPLR study site at the
213 Richardson Centre for Functional Foods and Nutraceuticals, University of Manitoba or
214 TMPLR's mobile research unit which travels to other areas of Winnipeg and southern Manitoba.
215 TMPLR's mobile research unit is a custom built 12-meter mobile lab which is equipped with
216 phlebotomy area, a dual-energy X-ray absorptiometer (DXA) and a bicycle ergometer with a
217 metabolic cart. During this visit, participants complete questionnaires, undergo various health
218 assessments, provide urine and fecal samples, and have fasting blood samples taken (**Figure 2,**
219 **Table 3**).

220 ***Questionnaires***

221 Questionnaires capture socio-demographic characteristics, personal and family medical history,
222 smoking (including electronic cigarette use), current diet (three Automated Self-Administered
223 24-h (ASA24) Dietary Assessment Tool recalls [18] , Mindful Eating Questionnaire [19], Diet
224 History Questionnaire [20] and The Three-Factor Eating Questionnaire [21]), alcohol
225 consumption, physical activities, frailty using the Modified Fried Criteria [22], stress, sleep
226 (Pittsburgh Sleep Quality Index [23]), cognition (Montreal Cognitive Assessment Questionnaire
227 [24]), and childhood retrospective circumstances (adapted from the US Panel Study on Income
228 Dynamics [25]).

229 ***Anthropometric assessment***

230 Weight is measured after participants change into lightweight scrub tops and bottoms, with shoes
231 removed, to the nearest 0.1 kg using a digital calibrated floor scale (7562EF, Taylor Precision
232 Products, Oak Brook, IL, USA). Height is measured, without shoes, to the nearest 0.1 cm using a
233 stadiometer (Model 206, SECA North America, Chino, CA, USA). BMI is calculated in kg/m².
234 Waist circumference is measured in triplicate, to the nearest 0.1 cm at the umbilicus, between the
235 last rib and the iliac crest using a fibreglass tape measure. Hip circumference is measured in
236 triplicate at the widest portion of the buttocks and hips using a fibreglass tape measure. Body
237 composition including fat mass, lean mass, percent body fat, visceral adipose tissue (VAT), and
238 bone mineral density (BMD) are assessed using dual-energy X-ray absorptiometry (DXA, Lunar
239 Prodigy Advance, GE Healthcare, Mississauga, ON, CAN) [26]. Scans are taken of the whole
240 body, femoral neck, L1-L4 of the spine, and the non-dominant forearm.

241 ***Clinical health assessment***

242 Participants' systolic and diastolic blood pressures are measured in triplicate, on the non-
243 dominant arm in a sitting position using a validated oscillometric blood pressure monitor
244 (BP760CAN, Omron, Burlington, ON, CAN). Participants are required to rest for 5-10 minutes
245 before taking the measurement. Pulse wave velocity and augmentation index are measured on the
246 non-dominant arm in a sitting position using a Mobil-O-Graph PWA Monitor and the HMS
247 Client Server Software (I.E.M GmbH, Stolberg, Germany) according to the manufacturer's
248 protocol on two consecutive days [27].

249 ***Collection of bio-specimens***

250 Blood, urine, and fecal samples are obtained from study participants (**Supplementary**
251 **protocols**). Fasting blood samples are collected on two consecutive days via venipuncture by
252 trained phlebotomists. Participants are asked to collect two urine samples at home; one sample is
253 obtained prior to going to bed, and a second of the first morning void upon waking up.

254 Participants also collect a fecal sample; they are provided a collection kit and instructed to
255 collect a single sample from 3 separate places on the stool using a spoon attached to the cap of
256 the collection tube. Participants are instructed to store the collected samples fecal in their
257 household -20°C freezer with a provided ice pack, and Urine samples in the fridge, until
258 transport back to the study center, using provided ice pack for temperature control, where they
259 are aliquoted and then stored at -80°C for future analysis [28].

260 ***Clinical chemistry in blood and urine***

261 Clinical chemistry, including lipid profile, glucose, insulin, and renal and liver profiles will be
262 measured via automated clinical chemistry analyzers (Cobas C111, C311 and e411, Roche
263 Diagnostics Laval, QC). Additional blood and urine biomarkers such as leptin, glucagon, and
264 melatonin will be measured via ligand binding assay (LBA) or enzyme-linked immunosorbent
265 assay (ELISA). Red blood cell and plasma fatty acids will be measured by gas chromatography
266 with flame ionization detections (GC-FID)[29]. Non-cholesterol sterols will be measured in
267 plasma using GC-FID and mass spectrometry (MS) [30]. Vitamin C concentrations in the blood
268 will be measure by high pressure liquid chromatography (HPLC) [31].

269 ***Microbiome analyses in fecal samples***

270 Fecal samples will be subjected to genomic DNA extraction (Zymo Research, CA, USA)
271 following the manufacturer's protocol. Experimental negative controls will be included in
272 extraction protocols to confirm the reliability and consistency of the extracted nucleic acid. The
273 V4 hypervariable region of 16S rRNA gene will be amplified, the sequencing library will be
274 generated as described previously [32] and sequenced at the Gut Microbiome Laboratory,
275 University of Manitoba. Samples will be multiplexed at the rate of 200 per run aiming for an
276 average sequencing depth of 50,000 sequences per sample. The sequencing data will be
277 deposited into the Sequence Read Archive (SRA) of NCBI (<http://www.ncbi.nlm.nih.gov/sra>)
278 and accession numbers will be provided for future access.

279 ***Deuterium oxide administration***

280 After the blood sample collection on day one, participants are given 0.7 grams of deuterium
281 oxide per kilogram of estimated body water to drink. Body water is estimated as body weight
282 (kg) x 0.60. This deuterium administration is used to enrich the body's water pool for the
283 assessment of fractional cholesterol and triglyceride synthesis rates [33-35].

284 ***Physical activity and capacity testing***

285 Physical activity level in TMPLR study participants is assessed using accelerometers (Actigraph
286 GTX3bt, Penscola. FL, USA) worn for 1 week [36, 37]. Muscle strength is measured using a
287 hand grip dynamometer. Cardiorespiratory fitness is assessed using a submaximal bike protocol
288 which includes heart rate monitoring, and a metabolic cart (VMAX Encore, Carefusion,
289 Unionville, Ont., Canada) to measure oxygen consumption and CO_2 output. Functional walking
290 ability is assessed using a 5-meter gait speed test. Additionally, depressive symptoms, obesity
291 history, frailty, low physical activity, and cognitive impairment are assessed by validated
292 questionnaires [38-40].

293 ***Sleep assessment***

294 Sleep in TMPLR study participants is measured objectively using accelerometers (Actigraph
295 GTX3bt, Penscola, FL, USA [41]) worn for a week and subjectively by questionnaire (Pittsburgh
296 Sleep Quality Index [23]). While there is a strong relationship between objective and subjective
297 sleep reports, TMPLR study is collecting both because discrepancies may provide important
298 clinical information reflecting early dysfunction [42, 43].

299 ***Dietary assessment***

300 Study participants complete the Canadian version of the Diet History Questionnaire (DHQ),
301 which estimates the intake of common food items and includes portion size and dietary
302 supplement questions. This questionnaire is on a TELEform for scanning data entry and creation
303 of the data files. Participants also complete three dietary recall surveys using the Automated
304 Self-Administered 24-hour Canada (ASA24®, NCI, Rockville, Maryland U.S. <http://asa24.ca/>)
305 dietary assessment tool, a web-based tool that enables multiple, automatically coded, self-
306 administered 24-hour recalls. Participants enrolled from March 2016 to February 2017 used the
307 ASA24-Canada-2014 edition; those enrolled after February 2017 used the ASA24-Canada-2016
308 edition. Both ASA24-Canada-2014 and ASA24-Canada-2016 use the same nutrient databases.

309 ***Early life experiences***

310 Early-life exposures spanning the critical time windows of fetal development, birth, infancy and
311 early childhood are documented in three ways: 1) through linkage with administrative health
312 records (described below), 2) by self-report, and 3) by maternal report. Mothers of TMPLR study
313 participants are asked to complete a TMPLR Mother's Questionnaire, adapted from the Nurses'
314 Health Study [44], capturing key pregnancy, birth, and postpartum events such as: method of
315 birth; gestational age and birth weight; socioeconomic status at birth; maternal pre-pregnancy
316 BMI and gestational weight gain; maternal smoking and diabetes during pregnancy; maternal
317 prenatal care; breastfeeding initiation, exclusivity and duration; stressful life events during
318 pregnancy and postpartum; and severe illness requiring hospitalization during infancy or early
319 childhood. Early childhood socioeconomic status [45, 46] and stressful life events [47, 48] are
320 also self-reported by TMPLR participants using the Childhood Retrospective Circumstances
321 Questionnaire, adapted from the US Panel Study of Income Dynamics [25].

322 **Data quality assurance and control**

323 Methods of data collection (questionnaires, anthropometric assessment, and clinical health
324 assessment) were standardized across the urban and mobile TMPLR study sites. Training of
325 TMPLR study staff involved in data collection and data entry is regularly refreshed and all staff
326 handling participant data are trained in compliance with the Manitoba Personal Health
327 Information Act (PHIA). All data are entered in the secure TMPLR study digital platform,
328 CREDIT (described below).

329 Questionnaire data entry is conducted via two methods. Questionnaires collected between March
330 2016 to October 2017 were collected on paper and entered manually by study staff.

331 Questionnaires collected from October 2017 onwards are entered directly by participants using a
332 digital questionnaire platform designed for TMPLR study using Clinical Research Electronic
333 Data InfrastrucTure (CREDIT) software (FunctionFour Inc., Winnipeg, MB, Canada), unless the
334 participant requests paper data collection. The digital platform allows for questionnaires to be
335 completed using a computer, tablet, or smartphone. Data fields have limits to check the logic and

336 reasonable range of responses. The manually entered data are checked for contradictory
337 responses in the double entry and manually corrected.

338 Any access of, or corrections made to, TMPLR study data in the digital platform are logged. To
339 protect the confidentiality of participants, all clinical data, questionnaire data and laboratory data
340 are kept secure and are identified only by a unique identifier. Participants' names, date of births,
341 addresses, and PHINs are kept in separately encrypted fields within the CREDIT software
342 platform. All data within the digital platform are encrypted at rest, using an encryption key that is
343 separate from the highly identifiable data listed above. Access to TMPLR study data and de-
344 encryption keys is only available for authorized research staff via a data manager. The electronic
345 data are stored on a server at the study site and are mirrored at a secure off-site location. A
346 TMPLR study data model has been created to help in visualizing the different types of data.

347 **(Supplementary figure 1)**

348 **Linkage to administrative health data**

349 At enrollment, TMPLR participants are asked to provide their PHIN and grant permission to link
350 their study data with administrative health records (including hospital discharge abstracts,
351 physician billing claims, and prescription records). These data are accessed through the Manitoba
352 Centre for Health Policy (MCHP) Population Research Data Repository [49] and linkage is
353 achieved using the PHIN, following the standard procedures established by the MCHP and the
354 Manitoba Health Information Privacy Committee. The data linkage is used to capture
355 retrospective information on early life as well as prospective information on numerous health
356 outcomes, including diagnosis of hypertension, T2D, CVD, and CKD.

357 **Statistical analyses**

358 Statistical analyses will be undertaken in consultation with biostatisticians from the George and
359 Fay Yee Centre for Healthcare Innovation (CHI) at the University of Manitoba. Lifestyle factors
360 will primarily be used as explanatory variables, with chronic disease biomarkers or disease
361 presence/absence as outcomes, in multivariable regression models. Moderating or mediating
362 effects of genetics, gut microbiome, clinical characteristics, socio-economic status, and
363 environmental factors will be explored. The potential confounding effects of health status and
364 healthcare use on variable relationships will be examined using techniques such as propensity
365 score or instrumental variable models [50-52].

367 Techniques appropriate for high-dimensional data will be adopted where needed. For example,
368 clustering of lifestyle risk factors will be examined using latent variable modeling techniques
369 (i.e., latent class analysis). Dimension reduction techniques for omics data, such as microbiome
370 and genetic markers, will be applied [53].

372 The bioinformatics and statistical analyses of microbiome data will be performed as described
373 previously [32] and will be updated based on recommendations and technology advancements
374 between now and processing of samples. Overall microbiota community structures, alpha
375 diversity metrics, and relative abundances of operational taxonomic units (OTUs), will be tested
376 for associations with lifestyle and health measures, with appropriate adjustment for multiple
377 comparisons.

378

379 Non-response bias may affect the validity of analyses for survey data, necessitating the use of
380 multiple imputation methods if the pattern of missing data is deemed to be ignorable [54]. For
381 non-ignorable missing data, selection and pattern mixture models will be examined in sensitivity
382 analyses [55]. Due to the use of non-random sampling there is a risk of selection bias; survey
383 weights and weighting of responses may be used to address this bias. Standardization or
384 adjustment techniques may be used to address bio-specimen measurement error bias [56].

385
386 Specialized methodological investigations will be conducted for: 1) psychometric analyses of
387 scales, including testing for differential item functioning and measurement invariance [57-59], 2)
388 development of chronic disease risk prediction models [60, 61], 3) techniques to evaluate the
389 quality of linked databases, including their accuracy, reliability, and completeness [62], 4) robust
390 statistical methods for the analysis of outcome measures with non-normal (e.g. skewed)
391 distributions [63, 64].

392 393 **Public engagement**

394 Three focus group, one for healthcare providers and two for general public, and a public forum
395 were held in the early design stages of this study to get input from Manitobans, on the study
396 design and recruitment strategies. A study advisory board was also formed, and meets on a bi-
397 annual basis. This advisory board includes healthcare providers, health researchers, and members
398 of the public. The board provides input regarding study recruitment, progress and conduct, and
399 will also provide input and suggestions regarding the dissemination of study results.

400 401 **Provision of clinical results to participants**

402 Individual results of the anthropomorphic measurements, blood pressure, pulse wave velocity,
403 augmentation index, body composition, bone density, full lipid profile, fasting blood glucose,
404 and renal and liver profile are provided to participants. They are referred to their primary care
405 providers for further management if their results are beyond clinical reference ranges.

406 407 **Ethics and dissemination**

408 Explicit informed consent is obtained from each individual prior to participation in the study.
409 Eligible participants are verbally informed by trained research personnel regarding the nature and
410 purpose of the study, given time to decide whether or not to participate, and have any questions
411 or concerns answered prior to consent and at any point throughout the study. All participants are
412 informed that they may withdraw from the study at any time without penalty and are reimbursed
413 for the portion of the study that they have completed up to that point.

414 Ethics approval has been obtained from the University of Manitoba Health Research Ethics
415 Board prior to participant recruitment (protocol# HS18951). The study protocol has also been
416 reviewed and approved by the Manitoba Health Information Privacy Committee in regards to the
417 collection and use of PHIN, The St. Boniface Hospital Research Review Committee in regards to
418 the processing of samples at the hospital, and the Winnipeg Regional Health Authority (WRHA)
419 Research Access and Approval Committee (RAAC), the Southern Health Research Ethics Board,
420 and the Interlake-Eastern Regional Health Authority Regional Ethics Committee, in regards to
421 the study taking place in those health regions.

422 Data analysis, release of results, and publication of initial manuscripts are scheduled for 2019.
423 Findings will be shared in peer-reviewed journals, and at regional, national, and international

423 scientific conferences. Data and findings will also be presented to healthcare policymakers
424 within Manitoba, to develop preventive strategies that reduce chronic conditions with the
425 intention of reducing healthcare costs. Funding applications for future clinical follow in this
426 study population will also be submitted starting in 2019.

427 **Discussion**

428 TMPLR study has been uniquely designed to provide cross-sectional, retrospective and
429 prospective observations that will improve our understanding of how lifestyle factors interact
430 with each other and additional factors such as genetics and the gut microbiome to influence
431 health and the risk of obesity, T2D, CVD, and CKD. The coordinated collection of lifestyle-
432 gene-environment-microbiota-health data including objective measurements such as DXA,
433 activity monitoring, stable isotopic tracer methodologies, and direct measurement of
434 physiological biomarkers, combined with the ability to retrospectively assess and prospectively
435 follow health outcomes in participants using administrative health records, represents an
436 unprecedented opportunity to collect data which can be used to improve chronic disease
437 prevention and management.

438 Due to the voluntary non-random recruitment of participants, there may be an under-
439 representation of those with lower health awareness, financial means, access, or time to
440 participate. Attempts to counteract this are implicit in the stratified recruitment design.
441 Comparisons between TMPLR study participants and general Manitoban population
442 demographics may allow assessment of potential selection biases. A healthy volunteer effect
443 may impact the ability to detect weak associations between lifestyle and disease risk, but this
444 may attenuate with longer follow-up using administrative health data.

445 In summary, TMPLR study will provide a unique platform of deeply phenotyped individuals that
446 will be used to explore the interactions between lifestyle factors that associate with the
447 development of, or protection from, obesity, hypertension, T2D, CVD, and CKD. The findings
448 from this research platform will subsequently be used to develop and test preventive and
449 restorative lifestyle and health strategies with the aim of improving the health and reducing
450 healthcare costs at the individual and population levels.

451 **Study status**

452 Data collection started in March 2016. As of the end of January 2018, data collection has been
453 completed for approximately 650 participants. The data collection and blood sampling are
454 expected to be completed by late 2018.

455 **Author contributions**

456 DSM and RCM developed the original concept of the study for the original grant application
457 with input from co-investigators. DSM prepared the drafts of the study protocol manuscript and
458 compiled feedback and changes from other authors. MG assisted in the preparation of the study
459 protocol manuscript. PF developed the branding for TMPLR study, and the manuscript figures
460 and tables. NM prepared the data model and was involved in the public engagement. SB (project
461 lead, indigenous health), HB (project lead, nutrition), JC, TAD (project lead, physical activity),
462 PKE (project lead, genetics), EK (project lead, gut microbiome), LML (project lead,
463 biostatistics), DEM (project lead, sleep), SBM, AR, NT, MBA (co-director and project lead,
464 developmental origins of chronic disease), and PJJ (senior-director) are study co-investigators,

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3 465 and were all involved in writing the original grant application. All authors have carefully read,
4 466 contributed to, and approved the final version of the study protocol manuscript.

5
6 467 **Funding statement**

7
8 468 This work is supported by a grant from Research Manitoba and the Province of Manitoba.
9 469 Financial and in-kind support for the TMPLR program was also provided by the Richardson
10 470 Centre for Functional Foods and Nutraceuticals, the George and Fay Yee Centre for Healthcare
11 471 Innovation, the University of Manitoba Office of Research Services, the University of Manitoba
12 472 Faculty of Agricultural and Food Sciences, and The Wellness Institute and the Chronic Disease
13 473 Innovation Centre at Seven Oaks Hospital. MG is funded by the Frederick Banting and Charles
14 474 Best Canada Graduate Scholarships-Master's. MBA holds a Canada Research Chair in the
15 475 Developmental Origins of Chronic Disease. PJJ holds a Canada Research Chair in Nutrition and
16 476 Functional Foods. These entities had no role in the design of the project.

17
18 477 **Competing interests statement**

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20 478 DSM, RCM, MG, SB, HB, JC, TAD, PKE, PF, NH, EK, LML, DEM, SBM, AR, MBA, and PJJ
21 479 have no competing interests to declare.

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645 **Figure 1. The Manitoba Personalized Lifestyle Research Study overview**

647 **Figure 2. The Manitoba Personalized Lifestyle Research Study participant schedule**

650 **Table 1. The Manitoba Personalize Lifestyle Research Study recruitment targets by strata**

Age	30-46 years							
	N=800							
Sex	400 Males				400 Females			
50% Male								
50% Female								
Geography	288 Urban		112 Rural		288 Urban		112 Rural	
72% Urban	males		males		females		females	
28% Rural								
BMI	116	172	45	67	116	172	45	67
40% Normal	Urban	Urban	Rural	Rural	Urban	Urban	Rural	Rural
(BMI <25)	males	males	males	males	females	females	females	females
60% Overweight	BMI	BMI	BMI	BMI	BMI	BMI	BMI	BMI

(BMI \geq 25)	<25	\geq 25	<25	\geq 25	<25	\geq 25	<25	\geq 25
+40 participants with severely reduced kidney function (eGFR <30 ml/min), 20 female, 20 male, with no set stratification based on BMI or geography								

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Table 2. The Manitoba Personalized Lifestyle Research (TMPLR) study estimated minimum detectable differences

Variable	Mean or median used	Standard deviation used	Minimum difference at 10% exposure (percentage of mean)	Minimum difference at 25% exposure (percentage of mean)	References
Body fat (%)	41.3% Females 27.8% Males	7.7% 6.6%	2.5% (6.0)	1.7% (4.0)	[17]
Lumbar bone mineral density (BMD; g/cm ²)	1.042 Females 1.058 Males	0.121 0.127	0.041 (3.8)	0.028 (2.6)	[65]
Glomerular filtration rate (GRF; ml/min per 1.73 m ²)	107.6	16.8	5.4 (5.0)	3.8 (3.5)	[66]
Systolic blood pressure (mmHg)	116	12	6.5 (5.6)	4.5 (3.9)	[67]
Fasting Glucose (mmol/L)	4.94	0.61	0.20 (4.0)	0.14 (2.8)	[67]
Fasting insulin (μ IU/mL)	7.83	7.50	2.40 (30)	1.67 (21%)	[68]

LDL cholesterol (mmol/L)	2.79	0.67	0.22 (7.8)	0.15 (5.4)	[67]
Waist circumference (cm)	80	10	3.2 (4)	2.2 (2.75)	[67]

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661 **Table 3. The Manitoba Personalized Lifestyle Research (TMPLR) study data, assessment**662 **tools and biological samples**

<i>Characteristic</i>	<i>Data</i>	<i>Method, Instrument or Source</i>
<i>Sociodemographic</i>	Date of birth, Sex, Ethnicity, Marital status	TMPLR study questionnaire
<i>Medical</i>	Personal medical history, Family medical history, Medication(s), Pregnancy history Cognition	TMPLR study questionnaire, Administrative health records Montreal Cognitive Assessment [24]
<i>Lifestyle</i>	Tobacco/smoking/vaping use, Alcohol use, Unintentional weight loss, Exhaustion, Depression	TMPLR study questionnaire
<i>Physical activity</i>	Frailty Physical activity Predicted VO2 max	Modified Fried Criteria [22] Paffenbarger physical activity index, Actigraphy [36, 37] Modified YMCA bike test with metabolic cart
<i>Nutrition</i>	Dietary patterns and habits	Mindful eating questionnaire[19], three-factor eating questionnaire [21], automated 24-hour dietary recall [18], Canadian dietary history questionnaire [20]
<i>Early life</i>	Childhood health, socio-demographic and socioeconomic status; Parental employment history Maternal: pregnancy events, obstetrical history, infant feeding	Childhood retrospective questionnaire, adapted from the US Panel Study on Income Dynamics[25] TMPLR Mother's retrospective childhood questionnaire, adapted from the Nurses Health Study [44]
<i>Socioeconomic</i>	Employment, Home ownership, Education attainment, Income	TMPLR study questionnaire
<i>Sleep and Stress</i>	Duration of sleep Sleep Quality Perception of stress, Daily life stressors	Actigraphy [41] Pittsburgh sleep quality index [23] Community-based stress and coping survey
<i>Anthropometric</i>	Height	Wall-mounted stadiometer

	Weight Waist circumference, Hip circumference Body fat, Lean mass, bone mineral density	Digital scale Tape measure Dual energy X-ray absorptiometry [26]
<i>Blood pressure</i>	Systolic & diastolic Pulse wave velocity, Augmentation index	Automated sphygmomanometer Mobil-O-Graph oscillometer [27]
<i>Biomarkers</i>	Blood clinical chemistry and biomarker assays Urinary clinical chemistry and biomarker assays Microbiome 16S RNA sequencing	Fasting blood samples Urine samples Fecal sample [32]

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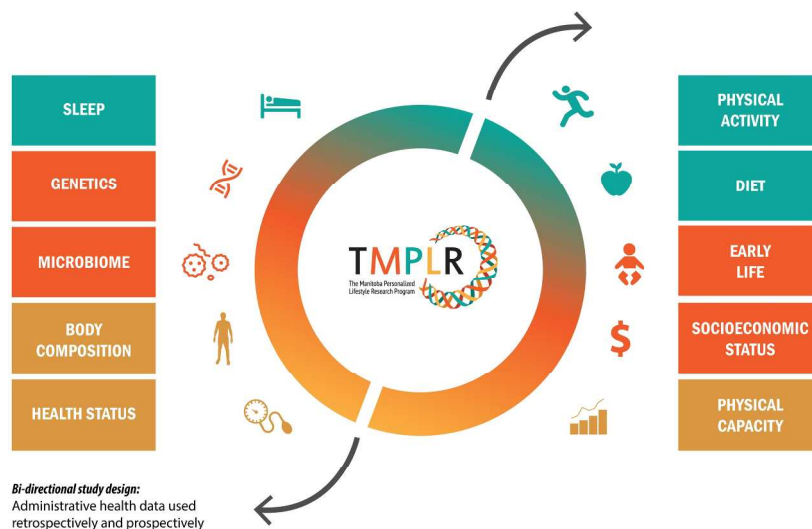


Figure 1. The Manitoba Personalized Lifestyle Research Study overview

230x130mm (300 x 300 DPI)

Review only



PARTICIPANT SCHEDULE

CONSENT PROCESS (completed before Day 1 activities)	
Day 1 (est. 2 hours)	
1	Collect link to administrative health records
2	Anthropometric measurements
3	PWA/PWV & blood pressure
4	Fasting blood samples
5	Oral administration of deuterium
6	Dual energy x-ray absorptiometry (DXA)
7	Fecal & urine sample kits
Day 2 (est. 2 hours)	
1	Fecal & urine collection
2	PWA/PWV & blood pressure
3	Fasting blood samples
4	Physical capacity testing
5	Sub-maximal cardiorespiratory fitness test
6	Start of activity monitoring (return accelerometer after 7 days of tracking)
Take home activities	
1	Questionnaires via website
2	Complete three automated 24-hour dietary recalls
18.02.12-01	

Figure 2. The Manitoba Personalized Lifestyle Research Study participant schedule

279x361mm (300 x 300 DPI)

TMPLR Data Model

Linking key: TMPLR ID #

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Data Type

Domain

Source

Clinical

Demographics (DM)

Anthropometric (AN)

Cardiovascular Function (CV)

Body Composition (BC)

PHIN

Frailty (FR)

Medical History (MH)

General Background (GB)

Lifestyle (LS)

Stress and Coping (SC)

Early Life Exposures (EL)

Nutrition (NU)

Sleep (SL)

Physical Activity (PA)

Fatty acid and Cholesterol Metabolism (FC)

Energy and Satiety Hormones (ES)

Diabetes and Glucose Metabolism (DG)

Liver Enzymes (LE)

Kidney Function (KF)

Immune Function (IF)

Lipidomic Profile (LP)

Genetic Profile (GP)

Biomarkers of Gut Microbiota (GM)

Clinical Report Form

Main Questionnaire

Supplementary Questionnaires

Accelerometers

Blood Sample

Urine Sample

Stool Sample

BMJ Open: first published as 10.1136/bmjopen-2018-023348 on 10 October 2019. Downloaded from <http://bmjopen.bmj.com/> on April 19, 2024 by guest. Protected by copyright.



The Manitoba Personalized Lifestyle Research (TMPLR) Study

Urine Sample Collection Instructions

Please follow these instructions for urine collection. Research personnel will provide you with 2 urine collection cups labeled with time (night and day) and your TMPLR Study ID Number.

- 1. Check your study ID on the collection tubes.** If the study ID is not correct, please correct it yourself, and inform a TMPLR clinical coordinator when you return the samples.
- 2. Collect urine before going to bed tonight in the cup labeled “night”. Please write down the date and time of the sample was collected.** Store the sample in the fridge in the Ziploc bag provided.
- 3. Collect urine from the first time you pee after getting up in the morning, in the cup labeled “day”. Please write down the date and time of the sample was collected.** Store your samples in the fridge in the Ziploc bag provided.
- 4. Please bring the urine samples with you on your day 2 visit.** TMPLR staff will collect the samples from you when you arrive.

If you have any questions, please contact the study coordinator Jeann Buenafe at tmplrtrial@umanitoba.ca or call 204-298-5483.

Thank you for your cooperation!

The Manitoba Personalized Lifestyle Research (TMPLR) Study

Stool Sample Collection Instructions

1. Freeze the ice packs provided by the study once you get home.
2. **Check your study ID** on the collection tubes (the two plastic tubes with blue cap). If the study ID is not correct, please correct it yourself, and inform a TMPLR clinical coordinator when you return the sample.
3. Empty your bladder. Flush toilet. Place the collection unit under the rear part of the toilet seat with the round side pointing towards the back.
4. Have a bowel movement. **Collect 2 samples, one in each plastic tube, from 3 different places of the stool** using the spoon attached to the cap of the collection tubes. **Fill each sample tube about one third of the tube with stool sample.**
5. Close the tube tightly. Place each tube in a Ziploc bag provided. **Write down the date and time of the bowel movement** on the bag. Discard the used collection unit.
6. Wrap the collection tubes with the frozen ice packs, and keep them in the paper bag provided. Keep the collected sample in the freezer.
7. Return the stool samples wrapped with the frozen ice packs on day 2 of the measurements, or as soon as you can. TMPLR staff will collect the sample from you in the paper bag when you arrive.

If you have any questions, feel free to contact the study coordinator Jeann Buenafe at tmplrtrial@umanitoba.ca or call 204-298-5483.



Collection Unit



Collection Tube with Spoon

The Manitoba Personalized Lifestyle Research (TMPLR) Study

Biospecimens collection

Blood samples will undergo analysis for numerous established and emerging health biomarkers, these include: total cholesterol, LDL-C, HDL-C, triglycerides, glucose, AST, ALT, insulin, glucagon-like peptide-1 (GLP-1), leptin, c-reactive protein (CRP), fatty acids, HbA1c, T-regs, serum creatinine, blood urea nitrogen (BUN), non-cholesterol sterols, adipokines, cytokines, vitamin C, fat soluble vitamins, and lipidomic and metabolomics profiling. Gut microbiota analysis will be performed on stool samples. The assessment of gut microbiota is critical as increasing evidence suggests that some of the health effects of physical activity, sleep, and nutrition may be exerted through or modified via the gut microbiota. Participants' DNA will be obtained to determine genetic variations associated with chronic condition risk factors and telomere length measurement.

Urine Collection

Participants will be invited to collect urine from the time subsequent to going to bed (last void at bedtime not collected), to the first morning void. Urine samples will be received on day 2 (see Urine Sample Collection Instructions). Urine samples will undergo analysis for glucose, albumin, creatinine, melatonin, total protein and metabolomics profiling.

Blood collection

Fasting blood samples will be collected on both days (Day 1 and Day 2); they will be identified by participants' ID and separated as indicated (Table 5). Participants should come in fasting state (at least for 12h) and shouldn't take any alcoholic beverage for at least 48h before each visit. A total of 60 mL of blood will be obtained from participants (Appendix 21). Blood will be drawn by a certified phlebotomist and/or a register nurse.

Stool collection

Participants will be asked to collect stool sample from a bowel movement. After this, they will take samples randomly from 3 different places of the stool. Sample will be given to research personnel at the beginning of second appointment. Research personnel will provide instruction to volunteers at the end of the first visit (see Stool Sample Collection Instructions).

The Manitoba Personalized Lifestyle Research (TMPLR) Study

Urine collection processing and collection instructions

Steps	Processing instructions
1	Receive urine sample and store it directly on 4 °C
2	Aliquot tubes should be labeled with participant ID
3	Number of labels required: 7 – 2.0 ml urine labels (if a urine sample was received)
4	If a urine sample is received proceed as follows: <ul style="list-style-type: none"> • Determine the volume of the urine • Pour some urine into a sterile container(to keep) • Aliquot urine into 2 -16 x 100 mm tubes and centrifuge
5	Aliquot as follows: 5 cryovials – 2.0 ml / vial (Seven Oaks) 2 cryovials – 2.0 ml / vial (McMillan)
6	Packaging of samples for transport: These samples must not thaw and must arrive frozen at the research lab Pack a transport box with ice packs and the frozen samples .Place the address label on the box. Ask the courier to return the transport box.



The Manitoba Personalized Lifestyle Research (TMPLR) Study

Blood sample processing and collection instructions

Sample	Blood collection tube	Tube volume	Processing instructions	Aliquoting instructions	Analysis	Date
Serum	Red/grey SST tube	1 x 4mL	<ol style="list-style-type: none"> Invert 5 times Room temp for 30 min Spin for 10 min @ 1000 x g 	<ol style="list-style-type: none"> Aliquot serum into cryovials¹ with brown² caps (0.5mL/tube) Store at -80°C 	Insulin Lipid profile Glucose CRP GLP-1	1, 2
Plasma	CPT tube (sodium heparin)	1 x 8 mL	<ol style="list-style-type: none"> Invert tube 8- 10 times Spin for 30 min @1500- 1800 RCF Resuspend by inverting After addition of PBS spin for 15 min @ 300 RCF Aspirate off as much supernatant without disturbing the pellet Repeat wash in 10mL PBS Resuspend pellet in 3mL freezing medium -10% DMSO (Sigma), 20% FCS (JRH Bioscience) in RPMI1640 (Gibco) 	<ol style="list-style-type: none"> Aliquot entire contents above the gel and transfer to 15 mL Falcon tube Add PBS (w/o Ca++ or Mg++) to make 15 mL Store 1mL aliquots in -70°C using a Cyro-1°C/min freezing container. 	T-Regulatory cells*	1
Plasma heparin	Green top (lithium heparin)	1x 4 mL	<ol style="list-style-type: none"> Invert 8 times Spin immediately for 10 min @1300 x g 	<ol style="list-style-type: none"> Aliquot plasma into cryovials with green³ caps (0.5mL/tube) Store all fractions at -80°C 	C-reactive protein	1, 2
RBC			<ol style="list-style-type: none"> Invert 8 times Spin immediately for 10 min @ 1300 x g 	<ol style="list-style-type: none"> Aliquot RBC into cryovials with red⁵ caps (0.5mL/tube) Store all fractions at -80°C 	Fatty Acid Analysis	1, 2
White blood cells Heparin			<ol style="list-style-type: none"> Invert 8 times Spin immediately for 10 min @ 1300 x g 	<ol style="list-style-type: none"> Aliquot WBC (buffy coat) in 1 (one) Cryo.s™ (RNase and DNase free 	DNA extraction/ Telomere length	1, 2

The Manitoba Personalized Lifestyle Research (TMPLR) Study

Plasma EDTA	Purple top (K2 EDTA)	1X 10 mL	1. Invert 8 times Spin immediately for 10 min @ 1300 x g 2. After addition of Methanol/ EDTA, spin @ 16,000g for 10 min. @ 1300 x g	1. Aliquot plasma into cryovials with yellow caps (1.0 mL/tube) ⁵ 2. Add to 1 plasma aliquot (0.5ML), 1 volume of sample to 4 volumes of 90% methanol/water/1 mM EDTA 3. Place on dry ice for 5 min 4. Store all fractions at -80°C	Ascorbic acid	1,
Plasma EDTA			1. Invert 8 times 2. Spin immediately for 10 min @ 1300 x g	1. Aliquot plasma in cryovials with purple caps (0.5ml/tube) ⁶ 2. Store all fractions at -80°C	Leptin Glucagon Oxidized phospholipids and oxylipins	1,2
Plasma EDTA			1. Invert 8 times 2. Spin immediately for 10 min @ 1300 x g	1. Aliquot RBC into cryovials with purple caps (0.5mL/tube) ⁵ 2. Store all fractions at -80°C	Non-cholesterol sterols	1,2
White blood cells EDTA			1. Invert 8 times 2. Spin immediately for 10 min @ 1300 x g	Aliquot WBC (buffy coat) in 1 (one) Cryo.s™ (RNase and DNase free vials) ⁴ 2. Store at -80°C	DNA extraction/ Telomere length	1,2

BMJ Open

The Manitoba Personalized Lifestyle Research (TMPLR) study protocol: a multi-centre bi-directional observational cohort study with administrative health record linkage investigating the interactions between lifestyle and health in Manitoba, Canada

Journal:	<i>BMJ Open</i>
Manuscript ID	bmjopen-2018-023318.R1
Article Type:	Protocol
Date Submitted by the Author:	27-Aug-2018
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Primary Subject Heading:	Nutrition and metabolism
Secondary Subject Heading:	Epidemiology, Genetics and genomics
Keywords:	EPIDEMIOLOGY, Health informatics < BIOTECHNOLOGY & BIOINFORMATICS, NUTRITION & DIETETICS, Physiology < BASIC SCIENCES

For peer review only

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The Manitoba Personalized Lifestyle Research (TMPLR) study protocol: A multi-centre bi-directional observational cohort study with administrative health record linkage investigating the interactions between lifestyle and health in Manitoba, Canada

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Keywords: nutrition, physical activity, sleep, chronic disease, genetics, microbiome

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Word Count:4244

For peer review only

80 Abstract

81 Introduction

82 Lifestyle factors, such as diet, physical activity and sleep, are associated with the development of
83 many chronic diseases. The objective of The Manitoba Personalized Lifestyle Research
84 (TMPLR) study is to understand how these lifestyle factors interact with each other and
85 additional factors, such as an individual's genetics and gut microbiome, to influence health.

86 Methods

87 An observational study of adults, with extensive phenotyping by objective health and lifestyle
88 assessments, and retrospective assessment of early life experiences, with retrospective and
89 prospective utilization of secondary data from administrative health records.

90 Study population

91 A planned non-random convenience sample of 840 Manitobans aged 30-46 recruited from the
92 general population, stratified by sex (equal males and females), body mass index (BMI; 60% of
93 participants with a BMI >25 kg/m²), and geography (25% from rural areas,). These stratifications
94 were selected based on Manitoba demographics.

95 Measurements

96 Lifestyle factors assessed will include dietary pattern, physical activity, cardiovascular fitness
97 and sleep. Additional factors such as medical history, socio-economic status, alcohol and tobacco
98 consumption, cognition, stress and anxiety, and early life experiences will also be documented.
99 A maternal survey will be performed. Body composition and bone density will be measured by
100 dual energy x-ray absorptiometry. Blood pressure, pulse wave velocity, and augmentation index
101 will be measured on two consecutive days. Chronic disease risk biomarkers will be measured in
102 blood and urine samples. DNA will be extracted for genetic analysis. A fecal sample will be
103 collected for microbiome analysis. Participants may provide their Manitoba Personal Health
104 Information Number (PHIN) to link their study data with administrative health records.

105 Ethics and dissemination

106 Ethics approval has been obtained from the University of Manitoba Health Research Ethics
107 Board (protocol # HS18951; 05/01/2016). Data analysis, release of results, and publication of
108 manuscripts are scheduled to start in late 2018. Additional information at www.TMPLR.ca.

109 **Clinicaltrials.gov** NCT#xxxxxx

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3 111 **Article Summary**

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5 112 **Strengths and limitations of this study**

6 113 The study is designed to capture extensive phenotyping of participants in the areas of diet,
7 114 physical activity and sleep, combined with genetic and gut microbiome profiles. The ability to
8 115 link these study data to healthcare usage data retrospectively and prospectively is a key strength
9 116 of the research (**Figure 1**). The use of a mobile research unit to access rural populations makes
10 117 the study unique as geographic setting can strongly influence health-related behaviors.

11 118 The study uses non-random convenience sampling for feasibility reasons, which can introduce
12 119 selection bias and limit generalizability; however, preset stratification based on Manitoba's
13 120 demographics on sex, BMI, and geography have been implemented so that our final study
14 121 population will be more representative. Another limitation is that some of the questionnaires
15 122 used in TMPLR have not previously been validated, or not validated in the specific TMPLR
16 123 study population. Finally, the study sample size of 840 individuals was not selected to power a
17 124 specific primary hypothesis; however, it will provide a one-of-a-kind research platform of
18 125 extensively phenotyped participants in which to investigate the associations between lifestyle
19 126 and chronic disease.
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128 **Introduction**

129 Manitoba is a province located in central Canada with a population of just over 1.2 million
130 people. Most Manitobans (~60%) live in Winnipeg, the largest city, with ~27% of the population
131 living in rural areas [1]. Approximately half of Manitobans are living with at least one of the
132 following chronic conditions: obesity, hypertension, type 2 diabetes (T2D), cardiovascular
133 disease (CVD), or chronic kidney disease (CKD) [2]. Additionally Manitoba has the highest
134 incidence and prevalence of end stage renal disease in Canada, partly because of its high burden
135 of diabetes [2]. The consequences of these chronic conditions is substantial and the financial
136 burden, both personally and societally, is enormous. In the province of Manitoba, which has a
137 universal healthcare system, over 40 percent of total provincial revenues are spent on healthcare
138 [3]. The burden of conditions including T2D and CKD is not unique to Manitoba [4, 5], therefore
139 the primary and secondary prevention of these chronic conditions is a major international health
140 research priority [6].

141 It is well established that diet, physical activity, and sleep influence health and mortality [7-10].
142 Evidence-based guidelines pertaining to nutrition, physical activity and sleep exist to educate the
143 public on healthy lifestyle choices. However, most current lifestyle guidelines follow a one-size-
144 fits-all format, even though they are intended for populations comprising individuals with
145 diverse and complex health circumstances and unique factors influencing their ability to follow
146 the guidelines. This format may be a contributing factor to the poor adherence to lifestyle
147 guidelines. For example, although most people are aware that physical activity is important for
148 health, only 15% of the Canadian population achieve the national recommendations [11].
149 Similarly, it is estimated that 50% of women and 70% of men in Canada have energy intakes that
150 exceed their energy needs, while 50% to 90% have deficiencies in calcium and vitamin D [12].

151 There is now an increasing interest in the creation of lifestyle strategies or guidelines for specific
152 sub-populations or groups of individuals with specific characteristics [13-15]. It is hoped that
153 such tailored recommendations will be more effective, and that barriers to healthy lifestyle
154 practices can be ameliorated through personalization. Current one-size-fits-all recommendations
155 and strategies may not be effective due to (1) significant inter-individual variability or (2) shared
156 circumstances, such as geography, sleep/wake pattern, or socio-economic status, of a particular
157 group.

158 We hypothesize that an individual's lifestyle will be influenced by socio-economic status and
159 geography, and will interact with their genotype and gut microbiota to affect health [16, 17].
160 Accordingly, The Manitoba Personalized Lifestyle Research (TMPLR) study will involve the
161 coordinated collection of data related to socio-economic status, geography, nutrition, physical
162 activity, sleep, early life experiences, and health systems usage, in conjunction with the analysis
163 of genetics, gut microbiota, and risk factors for chronic conditions such as obesity, hypertension,
164 T2D, CVD, and CKD. After establishing the baseline characteristics of this study cohort,
165 administrative health records will be used retrospectively to examine the developmental origins
166 of health and disease [18], and prospectively to track and investigate the development of chronic
167 disease in the future, starting at 5 years after the initial study is complete. Consent will be
168 obtained to contact study participants for further clinical assessments, contingent on future
169 funding.

170 Data from this study will provide an ideal opportunity for the exploration and potential discovery
171 of new interactive mechanisms through which lifestyle factors affect health. We will be looking

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3 172 to collaborate with other existing studies [19-21]with overlapping measures to replicate such
4 173 findings, or increase sample size.. Findings from this research may be useful in guiding both
5 174 clinical and health policy decisions, and will also facilitate the design and testing of personalized
6 175 health promotion strategies. For example, if we are able to identify interactions between lifestyle
7 176 factors and disease risk, such as a genetic variant that associates with short sleep to negatively
8 177 impact health, a follow-up study could be designed looking to improve sleep hygiene specifically
9 178 in the group with the risk variant.

11 12 179 **Methods**

13 180 **Design**

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15 181 This is an exploratory observational cohort study with retrospective and prospective utilization of
16 182 secondary data from administrative health records (**Figure 1**). The Strengthening the Reporting
17 183 of Observational Studies in Epidemiology (STROBE) guidelines were followed where applicable
18 184 in the development of this protocol manuscript [22].

19 185 **Setting**

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21 186 Urban (Winnipeg) and rural (Morden, Winkler, Carman, Steinbach) areas with road access in
22 187 southern Manitoba, Canada.

23 188 **Objectives of the study**

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25 189 The objective of this study is to explore the complex interactions that exist between lifestyle,
26 190 genetics, and gut microbiota, and how these relate to risk factors for chronic conditions,
27 191 especially obesity, hypertension T2D, CVD, and CKD in Manitoba.

28 192 **Inclusion and exclusion criteria**

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30 193 A sample of 800 Manitobans aged 30-46, stratified by sex, BMI, and geography (**Table 1**) are
31 194 being recruited. Participants must have lived in Manitoba for a minimum of 5 years. Women
32 195 who are pregnant or lactating are not eligible to participate. Additionally, because it is expected
33 196 that very few of the 800 Manitobans who join TMPLR study from the general public will have
34 197 reduced kidney function (eGFR <30 ml/min), 40 participants from Manitoba (20 female, 20
35 198 male, with no set stratification based on BMI or geography) who have severely reduced kidney
36 199 function are being recruited from the renal health clinic at Seven Oaks General Hospital
37 200 (SOGH), Winnipeg, MB. Therefore, the study has a recruitment goal of 840 participants.

38 201 **Recruitment**

39 202 Participants are recruited through the use of printed flyers, online advertisements purchased via
40 203 Google, Facebook, and Twitter ad platforms and social media accounts, appearances in local TV,
41 204 radio, and print media, and direct contact with community groups, such as churches, sports
42 205 leagues, and community clubs. All patients who receive care in the SOGH renal health clinic,
43 206 who are aged 30-46, have been living in Manitoba for a minimum of the last 5 years, and are
44 207 able to provide informed consent are approached to enroll in the study as well.

45 208 **Sample size**

46 209 The sample size of TMPLR study was selected based on considerations of feasibility of
47 210 recruitment, costs, and logistics. However, given established values from other sources [23] and
48 211 our anticipated sample size of 840 participants, we estimate that we will have an 80% power (5%
49 212 significance, 2-sided) to detect a minimum body fat difference of 2.5% for rare exposures (i.e.,

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3 213 experienced by 10% of participants, such as smoking) and 1.7% for more common exposures
4 214 (experienced by 25% of participants, such as meeting the Canadian recommended 150 minutes of
5 215 moderate-to-vigorous physical activity). Additional estimated minimum detectable differences
6 216 are presented in **Table 2**. These lower limits should allow for the detection of clinically
7 217 meaningful changes in these outcomes.
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11 219 **Data Collection and Assessments**

12 220 On two consecutive days, participants come to either the urban TMPLR study site at the
13 221 Richardson Centre for Functional Foods and Nutraceuticals, University of Manitoba or
14 222 TMPLR's mobile research unit which travels to other areas of Winnipeg and southern Manitoba.
15 223 TMPLR's mobile research unit is a custom built 12-meter mobile lab which is equipped with
16 224 phlebotomy area, a dual-energy X-ray absorptiometer (DXA) and a bicycle ergometer with a
17 225 metabolic cart. During this visit, participants complete questionnaires, undergo various health
18 226 assessments, provide urine and fecal samples, and have fasting blood samples taken (**Figure 2**,
19 227 **Table 3**). The same protocols at were followed at both sites.
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22 228 ***Questionnaires***

23 229 Questionnaires capture socio-demographic characteristics, personal and family medical history,
24 230 smoking (including electronic cigarette use), current diet (three Automated Self-Administered
25 231 24-h (ASA24) Dietary Assessment Tool recalls , Mindful Eating Questionnaire [24], Diet
26 232 History Questionnaire [25] and The Three-Factor Eating Questionnaire [26]), alcohol
27 233 consumption, physical activities, frailty using the Modified Fried Criteria [27], stress, sleep
28 234 (Pittsburgh Sleep Quality Index [28]), cognition (Montreal Cognitive Assessment Questionnaire
29 235 [29]), and childhood retrospective circumstances (adapted from the US Panel Study on Income
30 236 Dynamics [30]).
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33 237 ***Anthropometric assessment***

34 238 Weight is measured after participants change into lightweight scrub tops and bottoms, with shoes
35 239 removed, to the nearest 0.1 kg using a digital calibrated floor scale (7562EF, Taylor Precision
36 240 Products, Oak Brook, IL, USA). Height is measured, without shoes, to the nearest 0.1 cm using a
37 241 stadiometer (Model 206, SECA North America, Chino, CA, USA). BMI is calculated in kg/m^2 .
38 242 Waist circumference is measured in triplicate, to the nearest 0.1 cm at the umbilicus, between the
39 243 last rib and the iliac crest using a fibreglass tape measure. Hip circumference is measured in
40 244 triplicate at the widest portion of the buttocks and hips using a fibreglass tape measure. Body
41 245 composition including fat mass, lean mass, percent body fat, visceral adipose tissue (VAT), and
42 246 bone mineral density (BMD) are assessed using dual-energy X-ray absorptiometry (DXA, Lunar
43 247 Prodigy Advance, GE Healthcare, Mississauga, ON, CAN) [31]. Scans are taken of the whole
44 248 body, femoral neck, L1-L4 of the spine, and the non-dominant forearm.
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47 249 ***Clinical health assessment***

48 250 Participants' systolic and diastolic blood pressures are measured in triplicate, on the non-
49 251 dominant arm in a sitting position using a validated oscillometric blood pressure monitor
50 252 (BP760CAN, Omron, Burlington, ON, CAN). Participants are required to rest for 5-10 minutes
51 253 before taking the measurement. Pulse wave velocity and augmentation index are measured on the
52 254 non-dominant arm in a sitting position using a Mobil-O-Graph PWA Monitor and the HMS
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255 Client Server Software (I.E.M Gmbh, Stolberg, Germany) according to the manufacturer's
256 protocol on two consecutive days [32].

257 *Collection of bio-specimens*

258 Blood, urine, and fecal samples are obtained from study participants (**Supplementary**
259 **protocols**). Fasting blood samples are collected on two consecutive days via venipuncture by
260 trained phlebotomists. Two blood samples on consecutive days are required to undertake the
261 isotopic assessment of fractional cholesterol and triglyceride synthesis rates. Participants are
262 asked to collect two urine samples at home; one sample is obtained prior to going to bed, and a
263 second of the first morning void upon waking up. Participants also collect a fecal sample; they
264 are provided a collection kit and instructed to collect a single sample from 3 separate places on
265 the stool using a spoon attached to the cap of the collection tube. Participants are instructed to
266 store the collected samples fecal in their household -20°C freezer with a provided ice pack, and
267 urine samples in the fridge, until transport back to the study center, using provided ice pack for
268 temperature control, where they are aliquoted and then stored at -80°C for future analysis [33].

269 *Clinical chemistry in blood and urine*

270 Clinical chemistry, including lipid profile, glucose, insulin, and renal and liver profiles will be
271 measured via automated clinical chemistry analyzers (Cobas C111, C311 and e411, Roche
272 Diagnostics Laval, QC). Additional blood and urine biomarkers such as leptin, glucagon, and
273 melatonin will be measured via ligand binding assay (LBA) or enzyme-linked immunosorbent
274 assay (ELISA). Red blood cell and plasma fatty acids will be measured by gas chromatography
275 with flame ionization detections (GC-FID)[34]. Non-cholesterol sterols will be measured in
276 plasma using GC-FID and mass spectrometry (MS) [35]. Vitamin C concentrations in the blood
277 will be measure by high pressure liquid chromatography (HPLC) [36].

278 *Microbiome analyses in fecal samples*

279 Fecal samples will be subjected to genomic DNA extraction (Zymo Research, CA, USA)
280 following the manufacturer's protocol. Experimental negative controls will be included in
281 extraction protocols to confirm the reliability and consistency of the extracted nucleic acid. The
282 V4 hypervariable region of 16S rRNA gene will be amplified, the sequencing library will be
283 generated as described previously [37] and sequenced at the Gut Microbiome Laboratory,
284 University of Manitoba. Samples will be multiplexed at the rate of 200 per run aiming for an
285 average sequencing depth of 50,000 sequences per sample. The sequencing data will be
286 deposited into the Sequence Read Archive (SRA) of NCBI (<http://www.ncbi.nlm.nih.gov/sra>)
287 and accession numbers will be provided for future access.

288 *Deuterium oxide administration*

289 After the blood sample collection on day one, participants are given 0.7 grams of deuterium
290 oxide per kilogram of estimated body water to drink. Body water is estimated as body weight
291 (kg) x 0.60. This deuterium administration is used to enrich the body's water pool for the
292 assessment of fractional cholesterol and triglyceride synthesis rates [38-40].

293 *Physical activity and capacity testing*

Physical activity level in TMPLR study participants is assessed using accelerometers (Actigraph GTX3bt, Penscola. FL, USA) worn for 1 week [41, 42]. Muscle strength is measured using a hand grip dynamometer. Cardiorespiratory fitness is assessed using a submaximal bike protocol which includes heart rate monitoring, and a metabolic cart (VMAX Encore, Carefusion, Unionville, Ont., Canada) to measure oxygen consumption and CO₂ output. Functional walking ability is assessed using a 5-meter gait speed test. Additionally, depressive symptoms, obesity history, frailty, low physical activity, and cognitive impairment are assessed by validated questionnaires [43-45].

302 ***Sleep assessment***

Sleep in TMPLR study participants is measured objectively using accelerometers (Actigraph GTX3bt, Penscola. FL, USA [46]) worn for a week and subjectively by questionnaire (Pittsburgh Sleep Quality Index [28]). While there is a strong relationship between objective and subjective sleep reports, TMPLR study is collecting both because discrepancies may provide important clinical information reflecting early dysfunction [47, 48].

308 ***Dietary assessment***

Study participants complete the Canadian version of the Diet History Questionnaire (DHQ) [25], which estimates the intake of common food items and includes portion size and dietary supplement questions. This questionnaire is on a TELEform for scanning data entry and creation of the data files. Participants also complete the Mindful Eating Questionnaire [24] to assess awareness of the physical and emotional sensations associated with eating, and The Three-Factor Eating Questionnaire [26] to assess dietary restraint, disinhibition and hunger in relation to eating. Participants also complete three dietary recall surveys using the Automated Self-Administered 24-hour Canada (ASA24®, NCI, Rockville, Maryland U.S. <http://asa24.ca/>) [49] dietary assessment tool, a web-based tool that enables multiple, automatically coded, self-administered 24-hour recalls. Participants enrolled from March 2016 to February 2017 used the ASA24-Canada-2014 edition; those enrolled after February 2017 used the ASA24-Canada-2016 edition. Both ASA24-Canada-2014 and ASA24-Canada-2016 use the same nutrient databases.

321 ***Early life experiences***

Early-life exposures spanning the critical time windows of fetal development, birth, infancy and early childhood are documented in three ways: 1) through linkage with administrative health records (see Linkage to administrative health data), 2) by self-report, and 3) by maternal report. Administrative health data will provide method of birth, gestational age, birth weight, diagnosis codes for post-delivery hospitalization, and post-delivery drug prescriptions. Mothers of TMPLR study participants are asked to complete a TMPLR Mother's Questionnaire, adapted from the Nurses' Health Study [50], capturing key pregnancy, birth, and postpartum events such as method of birth; gestational age and birth weight; socioeconomic status at birth; maternal pre-pregnancy BMI and gestational weight gain; maternal smoking and diabetes during pregnancy; maternal prenatal care; breastfeeding initiation, exclusivity and duration; stressful life events during pregnancy and postpartum; and severe illness requiring hospitalization during infancy or early childhood. Early childhood socioeconomic status [51, 52] and stressful life events [53, 54] are also self-reported by TMPLR participants using the Childhood Retrospective Circumstances Questionnaire, adapted from the US Panel Study of Income Dynamics [30].

336 **Data quality assurance and control**

337 Methods of data collection (questionnaires, anthropometric assessment, and clinical health
338 assessment) were standardized across the urban and mobile TMPLR study sites. Training of
339 TMPLR study staff involved in data collection and data entry is regularly refreshed and all staff
340 handling participant data are trained in compliance with the Manitoba Personal Health
341 Information Act (PHIA). All data will be entered in the secure digital platform. A TMPLR study
342 data model has been created to help in visualizing the different types of data the digital platform
343 will contain. (**Supplementary figure 1**)

344 **Linkage to administrative health data**

345 At enrollment, TMPLR participants are asked to provide their PHIN and grant permission to link
346 their study data with administrative health records (including hospital discharge abstracts,
347 physician billing claims, and prescription records). These data are accessed through the Manitoba
348 Centre for Health Policy (MCHP) Population Research Data Repository [55] and linkage is
349 achieved using the PHIN, following the standard procedures established by the MCHP and the
350 Manitoba Health Information Privacy Committee. The data linkage is used to capture
351 retrospective information on early life as well as prospective information on numerous health
352 outcomes, including diagnosis of hypertension, T2D, CVD, and CKD.

353 **Statistical analyses**

354 Statistical analyses will be undertaken in consultation with biostatisticians from the George and
355 Fay Yee Centre for Healthcare Innovation (CHI) at the University of Manitoba. Lifestyle factors
356 will primarily be used as explanatory variables, with chronic disease biomarkers or disease
357 presence/absence as outcomes, in multivariable regression models. Moderating or mediating
358 effects of genetics, gut microbiome, clinical characteristics, socio-economic status, and
359 environmental factors will be explored. The potential confounding effects of health status and
360 healthcare use on variable relationships will be examined using techniques such as propensity
361 score or instrumental variable models [56-58].

362
363 Techniques appropriate for high-dimensional data will be adopted where needed. For example,
364 clustering of lifestyle risk factors will be examined using latent variable modeling techniques
365 (i.e., latent class analysis). Dimension reduction techniques for omics data, such as microbiome
366 and genetic markers, will be applied [59].

367
368 The bioinformatics and statistical analyses of microbiome data will be performed as described
369 previously [37] and will be updated based on recommendations and technology advancements
370 between now and the point of processing of samples. Overall microbiota community structures,
371 alpha diversity metrics, and relative abundances of operational taxonomic units (OTUs), will be
372 tested for associations with lifestyle and health measures, with appropriate adjustment for
373 multiple comparisons.

374
375 Non-response bias may affect the validity of analyses for survey data, necessitating the use of
376 multiple imputation methods if the pattern of missing data is deemed to be ignorable [60]. For
377 non-ignorable missing data, selection and pattern mixture models will be examined in sensitivity
378 analyses [61]. Due to the use of non-random sampling there is a risk of selection bias; survey
379 weights and weighting of responses may be used to address this bias. Standardization or
380 adjustment techniques may be used to address bio-specimen measurement error bias [62].

381

Specialized methodological investigations will be conducted for: 1) psychometric analyses of scales, including testing for differential item functioning and measurement invariance [63-65], 2) development of chronic disease risk prediction models [66, 67], 3) techniques to evaluate the quality of linked databases, including their accuracy, reliability, and completeness [68], 4) robust statistical methods for the analysis of outcome measures with non-normal (e.g. skewed) distributions [69, 70].

'Patient and Public Involvement' Three focus group, one for healthcare providers and two for general public, and a public forum were held in the early design stages of this study to obtain input from Manitobans, on the study design and recruitment strategies. A study advisory board was also formed, and meets on a bi-annual basis. This advisory board includes healthcare providers, health researchers, and members of the public. The board provides input regarding study recruitment, progress and conduct, and will also provide input and suggestions regarding the dissemination of study results.

Provision of clinical results to participants

Individual results of the anthropomorphic measurements, blood pressure, pulse wave velocity, augmentation index, body composition, bone density, full lipid profile, fasting blood glucose, and renal and liver profile are to be provided to participants. Participants are referred to their primary care providers for further management if their results are beyond clinical reference ranges. Participants will not be provided their genetic and microbiome information.

Ethics and dissemination

Explicit informed consent is obtained from each individual prior to participation in the study. Eligible participants are verbally informed by trained research personnel regarding the nature and purpose of the study, given time to decide whether or not to participate, and have any questions or concerns answered prior to consent and at any point throughout the study. All participants are informed that they may withdraw from the study at any time without penalty and are remunerated for the portion of the study that they have completed up to that point. The full remuneration for study participation is \$100 Canadian dollars, provided as cash or as a gift card.

Ethics approval has been obtained from the University of Manitoba Health Research Ethics Board prior to participant recruitment (protocol# HS18951). The study protocol has also been reviewed and approved by the Manitoba Health Information Privacy Committee in regards to the collection and use of PHIN, The St. Boniface Hospital Research Review Committee in regards to the processing of samples at the hospital, and the Winnipeg Regional Health Authority (WRHA) Research Access and Approval Committee (RAAC), the Southern Health Research Ethics Board, and the Interlake-Eastern Regional Health Authority Regional Ethics Committee, in regards to the study taking place in those health regions.

Data analysis, release of results, and publication of initial manuscripts are scheduled for 2019. Findings will be shared in peer-reviewed journals, and at regional, national, and international scientific conferences. Data and findings will also be presented to healthcare policymakers within Manitoba, to develop preventive strategies that reduce chronic conditions with the intention of reducing healthcare costs. Funding applications for future clinical follow in this study population have been submitted starting in 2017.

Discussion

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3 426 TMPLR study has been uniquely designed to provide cross-sectional, retrospective and
4 427 prospective observations that will improve our understanding of how lifestyle factors interact
5 428 with each other and additional factors such as genetics and the gut microbiome to influence
6 429 health and the risk of obesity, T2D, CVD, and CKD. The coordinated collection of lifestyle-
7 430 gene-environment-microbiota-health data, including objective measurements such as DXA,
8 431 activity monitoring, stable isotopic tracer methodologies, and direct measurement of
9 432 physiological biomarkers; combined with the ability to retrospectively assess and prospectively
10 433 follow health outcomes in participants using administrative health records, represents an
11 434 unprecedented opportunity to collect data which can be used to improve chronic disease
12 435 prevention and management.

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15 436 Due to the voluntary non-random recruitment of participants, there may be an under-
16 437 representation of those with lower health awareness, financial means, access, or time to
17 438 participate. Attempts to counteract this are implicit in the stratified recruitment design.
18 439 Comparisons between TMPLR study participants and general Manitoban population
19 440 demographics may allow assessment of potential selection biases. A healthy volunteer effect
20 441 may impact the ability to detect weak associations between lifestyle and disease risk, but this
21 442 may attenuate with longer follow-up using administrative health data.

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24 443 Given a projected sample size of 840 participants may be low for some of research questions that
25 444 will be investigated, therefore harmonization and linking of data across multiple cohorts may be
26 445 required. We will be looking to other studies which have undertaken overlapping measurements
27 446 in order to increase sample sizes. The Canadian Longitudinal Study on Aging [19], the Toronto
28 447 Nutrigenomics and Health [20], and The LifeLines DEEP [21] studies among others will be
29 448 approached regarding the potential of data harmonization and cross-replication. TMPLR study
30 449 will also be available to other researchers who are interested in collaboration or using the data for
31 450 cross-replication.

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33
34 451 In summary, TMPLR study will provide a unique platform of extensively phenotyped
35 452 individuals that will be used to explore the interactions between lifestyle factors that associate
36 453 with the development of, or protection from, obesity, hypertension, T2D, CVD, and CKD. The
37 454 findings from this research platform will subsequently be used to develop and test preventive and
38 455 restorative lifestyle and health strategies with the aim of improving the health and reducing
39 456 healthcare costs at the individual and population levels.

40 41 457 **Study status**

42 458 Data collection started in March 2016. As of the August 15th 2018, data collection has been
43 459 completed for over 800 participants.

44 45 460 **Acknowledgements**

46
47 461 The authors would to thank all the Manitobans who have participated in this study, without your
48 462 valuable contributions we would not be able to undertake this research. The authors would also
49 463 like to thank the Manitobans who took part in focus groups, and who joined the study advisory
50 464 board, for their important contributions to this study. Finally, the authors would like to
51 465 acknowledge the amazing staff involved in making TMPLR study a reality, in particular
52 466 Stephanie Jew, Sandra Castillo-San Juan, Jeann Buenafe, Meaghan Rempel, Katrina Cachero,
53 467 Mark Pinder, Eden Vergara and Kamlesh Patel.

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3 469 **Author contributions**

4
5 470 DSM and RCM developed the original concept of the study for the original grant application
6 471 with input from co-investigators. DSM prepared the drafts of the study protocol manuscript and
7 472 compiled feedback and changes from other authors. MG assisted in the preparation of the study
8 473 protocol manuscript. PF developed the branding for TMPLR study, and the manuscript figures
9 474 and tables. NM prepared the data model and was involved in the public engagement. SB (project
10 475 lead, indigenous health), HB (project lead, nutrition), JC, TAD (project lead, physical activity),
11 476 PKE (project lead, genetics), EK (project lead, gut microbiome), LML (project lead,
12 477 biostatistics), DEM (project lead, sleep), SBM, AR, NT, MBA (project lead, developmental
13 478 origins of chronic disease), and PJJ (Director) are study co-investigators, and were all involved
14 479 in writing the original grant application. All authors have carefully read, contributed to, and
15 480 approved the final version of the study protocol manuscript.

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17
18 481 **Funding statement**

19
20 482 This work is supported by a grant from Research Manitoba and the Province of Manitoba.
21 483 Financial and in-kind support for the TMPLR program was also provided by the Richardson
22 484 Centre for Functional Foods and Nutraceuticals, the George and Fay Yee Centre for Healthcare
23 485 Innovation, the University of Manitoba Office of Research Services, the University of Manitoba
24 486 Faculty of Agricultural and Food Sciences, and The Wellness Institute and the Chronic Disease
25 487 Innovation Centre at Seven Oaks Hospital. MG is funded by the Frederick Banting and Charles
26 488 Best Canada Graduate Scholarships-Master's. MBA holds a Canada Research Chair in the
27 489 Developmental Origins of Chronic Disease. PJJ holds a Canada Research Chair in Nutrition and
28 490 Functional Foods. These entities had no role in the design of the project.

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31 491 **Competing interests statement**

32 492 DSM, RCM, MG, SB, HB, JC, TAD, PKE, PF, NH, EK, LML, DEM, SBM, AR, MBA, and PJJ
33 493 have no competing interests to declare.

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37 673 **Figure 1. The Manitoba Personalized Lifestyle Research Study overview**

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39 675 **Figure 2. The Manitoba Personalized Lifestyle Research Study participant schedule**

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42 678 **Table 1. The Manitoba Personalize Lifestyle Research Study recruitment targets by strata**

Age	30-46 years	
	N=800	
Sex	400 Males	400 Females

50% Male 50% Female								
Geography 72% Urban 28% Rural	288 Urban males		112 Rural males		288 Urban females		112 Rural females	
BMI 40% Normal (BMI <25) 60% Overweight (BMI ≥ 25)	116 Urban males BMI <25	172 Urban males BMI ≥ 25	45 Rural males BMI <25	67 Rural males BMI ≥ 25	116 Urban females BMI <25	172 Urban females BMI ≥ 25	45 Rural females BMI <25	67 Rural females BMI ≥ 25
+40 participants with severely reduced kidney function (eGFR <30 ml/min), 20 female, 20 male, with no set stratification based on BMI or geography								

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Table 2. The Manitoba Personalized Lifestyle Research (TMPLR) study estimated minimum detectable differences

Variable	Mean or median used	Standard deviation used	Minimum difference at 10% exposure (percentage of mean)	Minimum difference at 25% exposure (percentage of mean)	References
Body fat (%)	41.3% Females 27.8% Males	7.7% 6.6%	2.5% (6.0)	1.7% (4.0)	[23]
Lumbar bone mineral density (BMD; g/cm ²)	1.042 Females 1.058 Males	0.121 0.127	0.041 (3.8)	0.028 (2.6)	[71]
Glomerular filtration rate (GRF; ml/min per 1.73 m ²)	107.6	16.8	5.4 (5.0)	3.8 (3.5)	[72]

Systolic blood pressure (mmHg)	116	12	6.5 (5.6)	4.5 (3.9)	[73]
Fasting Glucose (mmol/L)	4.94	0.61	0.20 (4.0)	0.14 (2.8)	[73]
Fasting insulin (μ IU/mL)	7.83	7.50	2.40 (30)	1.67 (21%)	[74]
LDL cholesterol (mmol/L)	2.79	0.67	0.22 (7.8)	0.15 (5.4)	[73]
Waist circumference (cm)	80	10	3.2 (4)	2.2 (2.75)	[73]

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689 **Table 3. The Manitoba Personalized Lifestyle Research (TMPLR) study data, assessment**690 **tools and biological samples**

<i>Characteristic</i>	<i>Data</i>	<i>Method, Instrument or Source</i>
<i>Sociodemographic</i>	Date of birth, Sex, Ethnicity, Marital status	TMPLR study questionnaire
<i>Medical</i>	Personal medical history, Family medical history, Medication(s), Pregnancy history Cognition	TMPLR study questionnaire, Administrative health records Montreal Cognitive Assessment [29]
<i>Lifestyle</i>	Tobacco/smoking/vaping use, Alcohol use, Unintentional weight loss, Exhaustion, Depression	TMPLR study questionnaire
<i>Physical activity</i>	Frailty	Modified Fried Criteria [27]
	Physical activity	Paffenbarger physical activity index, Actigraphy [41, 42]
	Predicted VO ₂ max	Modified YMCA bike test with metabolic cart
<i>Nutrition</i>	Dietary patterns and habits	Mindful eating questionnaire[24], three-factor eating questionnaire [26], automated 24-hour dietary recall [49], Canadian dietary history questionnaire [25]
<i>Early life</i>	Childhood health, socio-demographic and socioeconomic status; Parental employment history	Childhood retrospective questionnaire, adapted from the US Panel Study on Income Dynamics[30]

	Maternal: pregnancy events, obstetrical history, infant feeding	TMPLR Mother's retrospective childhood questionnaire, adapted from the Nurses Health Study [50]
<i>Socioeconomic</i>	Employment, Home ownership, Education attainment, Income	TMPLR study questionnaire
<i>Sleep and Stress</i>	Duration of sleep Sleep Quality Perception of stress, Daily life stressors	Actigraphy [46] Pittsburgh sleep quality index [28] Community-based stress and coping survey
<i>Anthropometric</i>	Height Weight Waist circumference, Hip circumference Body fat, Lean mass, bone mineral density	Wall-mounted stadiometer Digital scale Tape measure Dual energy X-ray absorptiometry [31]
<i>Blood pressure</i>	Systolic & diastolic Pulse wave velocity, Augmentation index	Automated sphygmomanometer Mobil-O-Graph oscillometer [32]
<i>Biomarkers</i>	Blood clinical chemistry and biomarker assays Urinary clinical chemistry and biomarker assays Microbiome 16S RNA sequencing	Fasting blood samples Urine samples Fecal sample [37]

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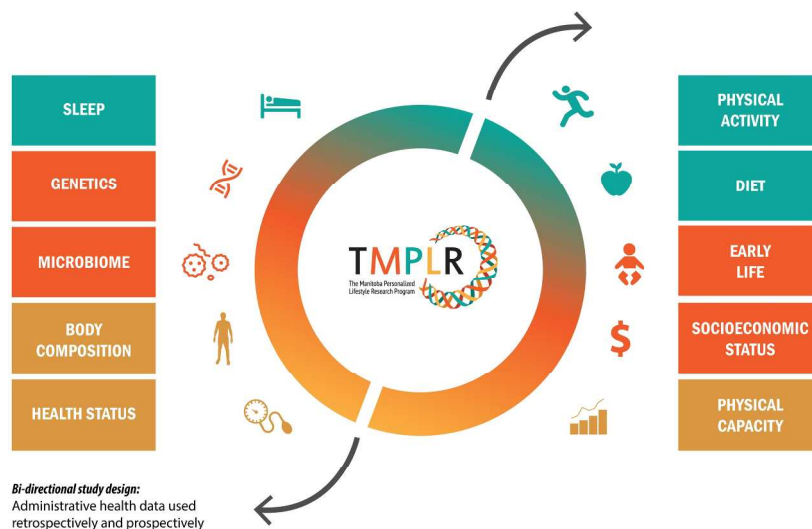


Figure 1. The Manitoba Personalized Lifestyle Research Study overview

230x130mm (300 x 300 DPI)

Review only



PARTICIPANT SCHEDULE

CONSENT PROCESS (completed before Day 1 activities)	
Day 1 (est. 2 hours)	
1	Collect link to administrative health records
2	Anthropometric measurements
3	PWA/PWV & blood pressure
4	Fasting blood samples
5	Oral administration of deuterium
6	Dual energy x-ray absorptiometry (DXA)
7	Fecal & urine sample kits
Day 2 (est. 2 hours)	
1	Fecal & urine collection
2	PWA/PWV & blood pressure
3	Fasting blood samples
4	Physical capacity testing
5	Sub-maximal cardiorespiratory fitness test
6	Start of activity monitoring (return accelerometer after 7 days of tracking)
Take home activities	
1	Questionnaires via website
2	Complete three automated 24-hour dietary recalls
18.02.12-01	

Figure 2. The Manitoba Personalized Lifestyle Research Study participant schedule

279x361mm (300 x 300 DPI)

TMPLR Data Model

Linking key: TMPLR ID #

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Data Type

Domain

Source

Clinical

Demographics (DM)

Anthropometric (AN)

Cardiovascular Function (CV)

Body Composition (BC)

PHIN

Frailty (FR)

Medical History (MH)

General Background (GB)

Lifestyle (LS)

Stress and Coping (SC)

Early Life Exposures (EL)

Nutrition (NU)

Sleep (SL)

Physical Activity (PA)

Fatty acid and Cholesterol Metabolism (FC)

Energy and Satiety Hormones (ES)

Diabetes and Glucose Metabolism (DG)

Liver Enzymes (LE)

Kidney Function (KF)

Immune Function (IF)

Lipidomic Profile (LP)

Genetic Profile (GP)

Biomarkers of Gut Microbiota (GM)

Clinical Report Form

Main Questionnaire

Supplementary Questionnaires

Accelerometers

Blood Sample

Urine Sample

Stool Sample

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The Manitoba Personalized Lifestyle Research (TMPLR) Study

Urine Sample Collection Instructions

Please follow these instructions for urine collection. Research personnel will provide you with 2 urine collection cups labeled with time (night and day) and your TMPLR Study ID Number.

- 1. Check your study ID on the collection tubes.** If the study ID is not correct, please correct it yourself, and inform a TMPLR clinical coordinator when you return the samples.
- 2. Collect urine before going to bed tonight in the cup labeled “night”. Please write down the date and time of the sample was collected.** Store the sample in the fridge in the Ziploc bag provided.
- 3. Collect urine from the first time you pee after getting up in the morning, in the cup labeled “day”. Please write down the date and time of the sample was collected.** Store your samples in the fridge in the Ziploc bag provided.
- 4. Please bring the urine samples with you on your day 2 visit.** TMPLR staff will collect the samples from you when you arrive.

If you have any questions, please contact the study coordinator Jeann Buenafe at tmplrtrial@umanitoba.ca or call 204-298-5483.

Thank you for your cooperation!

The Manitoba Personalized Lifestyle Research (TMPLR) Study

Stool Sample Collection Instructions

1. Freeze the ice packs provided by the study once you get home.
2. **Check your study ID** on the collection tubes (the two plastic tubes with blue cap). If the study ID is not correct, please correct it yourself, and inform a TMPLR clinical coordinator when you return the sample.
3. Empty your bladder. Flush toilet. Place the collection unit under the rear part of the toilet seat with the round side pointing towards the back.
4. Have a bowel movement. **Collect 2 samples, one in each plastic tube, from 3 different places of the stool** using the spoon attached to the cap of the collection tubes. **Fill each sample tube about one third of the tube with stool sample.**
5. Close the tube tightly. Place each tube in a Ziploc bag provided. **Write down the date and time of the bowel movement** on the bag. Discard the used collection unit.
6. Wrap the collection tubes with the frozen ice packs, and keep them in the paper bag provided. Keep the collected sample in the freezer.
7. Return the stool samples wrapped with the frozen ice packs on day 2 of the measurements, or as soon as you can. TMPLR staff will collect the sample from you in the paper bag when you arrive.

If you have any questions, feel free to contact the study coordinator Jeann Buenafe at tmplrtrial@umanitoba.ca or call 204-298-5483.



Collection Unit



Collection Tube with Spoon

The Manitoba Personalized Lifestyle Research (TMPLR) Study

Biospecimens collection

Blood samples will undergo analysis for numerous established and emerging health biomarkers, these include: total cholesterol, LDL-C, HDL-C, triglycerides, glucose, AST, ALT, insulin, glucagon-like peptide-1 (GLP-1), leptin, c-reactive protein (CRP), fatty acids, HbA1c, T-regs, serum creatinine, blood urea nitrogen (BUN), non-cholesterol sterols, adipokines, cytokines, vitamin C, fat soluble vitamins, and lipidomic and metabolomics profiling. Gut microbiota analysis will be performed on stool samples. The assessment of gut microbiota is critical as increasing evidence suggests that some of the health effects of physical activity, sleep, and nutrition may be exerted through or modified via the gut microbiota. Participants' DNA will be obtained to determine genetic variations associated with chronic condition risk factors and telomere length measurement.

Urine Collection

Participants will be invited to collect urine from the time subsequent to going to bed (last void at bedtime not collected), to the first morning void. Urine samples will be received on day 2 (see Urine Sample Collection Instructions). Urine samples will undergo analysis for glucose, albumin, creatinine, melatonin, total protein and metabolomics profiling.

Blood collection

Fasting blood samples will be collected on both days (Day 1 and Day 2); they will be identified by participants' ID and separated as indicated (Table 5). Participants should come in fasting state (at least for 12h) and shouldn't take any alcoholic beverage for at least 48h before each visit. A total of 60 mL of blood will be obtained from participants (Appendix 21). Blood will be drawn by a certified phlebotomist and/or a register nurse.

Stool collection

Participants will be asked to collect stool sample from a bowel movement. After this, they will take samples randomly from 3 different places of the stool. Sample will be given to research personnel at the beginning of second appointment. Research personnel will provide instruction to volunteers at the end of the first visit (see Stool Sample Collection Instructions).

The Manitoba Personalized Lifestyle Research (TMPLR) Study

Urine collection processing and collection instructions

Steps	Processing instructions
1	Receive urine sample and store it directly on 4 °C
2	Aliquot tubes should be labeled with participant ID
3	Number of labels required: 7 – 2.0 ml urine labels (if a urine sample was received)
4	If a urine sample is received proceed as follows: <ul style="list-style-type: none"> • Determine the volume of the urine • Pour some urine into a sterile container(to keep) • Aliquot urine into 2 -16 x 100 mm tubes and centrifuge
5	Aliquot as follows: 5 cryovials – 2.0 ml / vial (Seven Oaks) 2 cryovials – 2.0 ml / vial (McMillan)
6	Packaging of samples for transport: These samples must not thaw and must arrive frozen at the research lab Pack a transport box with ice packs and the frozen samples .Place the address label on the box. Ask the courier to return the transport box.



The Manitoba Personalized Lifestyle Research (TMPLR) Study

Blood sample processing and collection instructions

Sample	Blood collection tube	Tube volume	Processing instructions	Aliquoting instructions	Analysis	Date
Serum	Red/grey SST tube	1 x 4mL	<ol style="list-style-type: none"> Invert 5 times Room temp for 30 min Spin for 10 min @ 1000 x g 	<ol style="list-style-type: none"> Aliquot serum into cryovials¹ with brown² caps (0.5mL/tube) Store at -80°C 	Insulin Lipid profile Glucose CRP GLP-1	1, 2
Plasma	CPT tube (sodium heparin)	1 x 8 mL	<ol style="list-style-type: none"> Invert tube 8- 10 times Spin for 30 min @1500- 1800 RCF Resuspend by inverting After addition of PBS spin for 15 min @ 300 RCF Aspirate off as much supernatant without disturbing the pellet Repeat wash in 10mL PBS Resuspend pellet in 3mL freezing medium -10% DMSO (Sigma), 20% FCS (JRH Bioscience) in RPMI1640 (Gibco) 	<ol style="list-style-type: none"> Aliquot entire contents above the gel and transfer to 15 mL Falcon tube Add PBS (w/o Ca++ or Mg++) to make 15 mL Store 1mL aliquots in -70°C using a Cyro-1°C/min freezing container. 	T-Regulatory cells*	1
Plasma heparin	Green top (lithium heparin)	1x 4 mL	<ol style="list-style-type: none"> Invert 8 times Spin immediately for 10 min @1300 x g 	<ol style="list-style-type: none"> Aliquot plasma into cryovials with green³ caps (0.5mL/tube) Store all fractions at -80°C 	C-reactive protein	1, 2
RBC			<ol style="list-style-type: none"> Invert 8 times Spin immediately for 10 min @ 1300 x g 	<ol style="list-style-type: none"> Aliquot RBC into cryovials with red⁵ caps (0.5mL/tube) Store all fractions at -80°C 	Fatty Acid Analysis	1, 2
White blood cells Heparin			<ol style="list-style-type: none"> Invert 8 times Spin immediately for 10 min @ 1300 x g 	<ol style="list-style-type: none"> Aliquot WBC (buffy coat) in 1 (one) Cryo.s™ (RNase and DNase free 	DNA extraction/ Telomere length	1, 2

The Manitoba Personalized Lifestyle Research (TMPLR) Study

Plasma EDTA	Purple top (K2 EDTA)	1X 10 mL	1. Invert 8 times Spin immediately for 10 min @ 1300 x g 2. After addition of Methanol/ EDTA, spin @ 16,000g for 10 min. @ 1300 x g	1. Aliquot plasma into cryovials with yellow caps (1.0 mL/tube) ⁵ 2. Add to 1 plasma aliquot (0.5ml), 1 volume of sample to 4 volumes of 90% methanol/water/1 mM EDTA 3. Place on dry ice for 5 min 4. Store all fractions at -80°C	Ascorbic acid	1,
Plasma EDTA			1. Invert 8 times 2. Spin immediately for 10 min @ 1300 x g	1. Aliquot plasma in cryovials with purple caps (0.5ml/tube) ⁶ 2. Store all fractions at -80°C	Leptin Glucagon Oxidized phospholipids and oxylipins	1,2
Plasma EDTA			1. Invert 8 times 2. Spin immediately for 10 min @ 1300 x g	1. Aliquot RBC into cryovials with purple caps (0.5ml/tube) ⁵ 2. Store all fractions at -80°C	Non-cholesterol sterols	1,2
White blood cells EDTA			1. Invert 8 times 2. Spin immediately for 10 min @ 1300 x g	Aliquot WBC (buffy coat) in 1 (one) Cryo.s™ (RNase and DNase free vials) ⁴ 2. Store at -80°C	DNA extraction/ Telomere length	1,2

BMJ Open

The Manitoba Personalized Lifestyle Research (TMPLR) study protocol: a multi-centre bi-directional observational cohort study with administrative health record linkage investigating the interactions between lifestyle and health in Manitoba, Canada

Journal:	<i>BMJ Open</i>
Manuscript ID	bmjopen-2018-023318.R2
Article Type:	Protocol
Date Submitted by the Author:	19-Sep-2018
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	University of Manitoba; University of Manitoba, Department of Food and Human Nutritional Sciences
Primary Subject Heading:	Nutrition and metabolism
Secondary Subject Heading:	Epidemiology, Genetics and genomics
Keywords:	EPIDEMIOLOGY, Health informatics < BIOTECHNOLOGY & BIOINFORMATICS, NUTRITION & DIETETICS, Physiology < BASIC SCIENCES

For peer review only

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The Manitoba Personalized Lifestyle Research (TMPLR) study protocol: A multi-centre bi-directional observational cohort study with administrative health record linkage investigating the interactions between lifestyle and health in Manitoba, Canada

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Keywords: nutrition, physical activity, sleep, chronic disease, genetics, microbiome

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Word Count:3855

For peer review only

80 Abstract

81 Introduction

82 Lifestyle factors, such as diet, physical activity and sleep, are associated with the development of
83 many chronic diseases. The objective of The Manitoba Personalized Lifestyle Research
84 (TMPLR) study is to understand how these lifestyle factors interact with each other and other
85 factors, such as an individual's genetics and gut microbiome, to influence health.

86 Methods

87 An observational study of adults, with extensive phenotyping by objective health and lifestyle
88 assessments, and retrospective assessment of early life experiences, with retrospective and
89 prospective utilization of secondary data from administrative health records.

90 Study population

91 A planned non-random convenience sample of 840 Manitobans aged 30-46 recruited from the
92 general population, stratified by sex (equal males and females), body mass index (BMI; 60% of
93 participants with a BMI >25 kg/m²), and geography (25% from rural areas,). These stratifications
94 were selected based on Manitoba demographics.

95 Measurements

96 Lifestyle factors assessed will include dietary pattern, physical activity, cardiovascular fitness
97 and sleep. Factors such as medical history, socio-economic status, alcohol and tobacco
98 consumption, cognition, stress and anxiety, and early life experiences will also be documented.
99 A maternal survey will be performed. Body composition and bone density will be measured by
100 dual energy x-ray absorptiometry. Blood pressure, pulse wave velocity, and augmentation index
101 will be measured on two consecutive days. Chronic disease risk biomarkers will be measured in
102 blood and urine samples. DNA will be extracted for genetic analysis. A fecal sample will be
103 collected for microbiome analysis. Participants may provide their Manitoba Personal Health
104 Information Number (PHIN) to link their study data with administrative health records.

105 Ethics and dissemination

106 Ethics approval has been obtained from the University of Manitoba Health Research Ethics
107 Board (protocol # HS18951; 05/01/2016). Data analysis, release of results, and publication of
108 manuscripts are scheduled to start in early 2019. Additional information at www.TMPLR.ca.

109 **Clinicaltrials.gov NCT03674957**

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3 111 **Article Summary**

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5 112 **Strengths and limitations of this study**

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7 113 • The study is designed to capture extensive phenotyping of participants in the areas of
8 114 diet, physical activity and sleep, genetic and gut microbiome profiles, and healthcare
9 115 usage data linkage.
- 10 116 • The use of a mobile research unit to access rural populations makes the study unique as
11 117 geographic setting can strongly influence health-related behaviors. The study uses non-
12 118 random convenience sampling for feasibility reasons, which can introduce selection bias
13 119 and limit generalizability.
- 14 120 • Some of the questionnaires used in TMPLR have not previously been validated, or not
15 121 validated in the specific TMPLR study population.
- 16 122 • The study sample size of 840 individuals was not selected to power a specific primary
17 123 hypothesis and therefore should be considered exploratory in nature.
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125 **Introduction**

126 Manitoba is a province located in central Canada with a population of just over 1.2 million
127 people. Most Manitobans (~60%) live in Winnipeg, the largest city, with ~27% of the population
128 living in rural areas [1]. Approximately half of Manitobans are living with at least one of the
129 following chronic conditions: obesity, hypertension, type 2 diabetes (T2D), cardiovascular
130 disease (CVD), or chronic kidney disease (CKD) [2]. Additionally Manitoba has the highest
131 incidence and prevalence of end stage renal disease in Canada, partly because of its high burden
132 of diabetes [2]. The consequences of these chronic conditions is substantial and the financial
133 burden, both personally and societally, is enormous. In the province of Manitoba, which has a
134 universal healthcare system, over 40 percent of total provincial revenues are spent on healthcare
135 [3]. The burden of conditions including T2D and CKD is not unique to Manitoba [4, 5], therefore
136 the primary and secondary prevention of these chronic conditions is a major international health
137 research priority [6].

138 It is well established that diet, physical activity, and sleep influence health and mortality [7-10].
139 Evidence-based guidelines pertaining to nutrition, physical activity and sleep exist to educate the
140 public on healthy lifestyle choices. However, most current lifestyle guidelines follow a one-size-
141 fits-all format, even though they are intended for populations comprising individuals with
142 diverse and complex health circumstances and unique factors influencing their ability to follow
143 the guidelines. This format may be a contributing factor to the poor adherence to lifestyle
144 guidelines. For example, although most people are aware that physical activity is important for
145 health, only 15% of the Canadian population achieve the national recommendations [11].
146 Similarly, it is estimated that 50% of women and 70% of men in Canada have energy intakes that
147 exceed their energy needs, while 50% to 90% have deficiencies in calcium and vitamin D [12].

148 There is now an increasing interest in the creation of lifestyle strategies or guidelines for specific
149 sub-populations or groups of individuals with specific characteristics [13-15]. It is hoped that
150 such tailored recommendations will be more effective, and that barriers to healthy lifestyle
151 practices can be ameliorated through personalization. Current one-size-fits-all recommendations
152 and strategies may not be effective due to (1) significant inter-individual variability or (2) shared
153 circumstances, such as geography, sleep/wake pattern, or socio-economic status, of a particular
154 group.

155 We hypothesize that an individual's lifestyle will be influenced by socio-economic status and
156 geography, and will interact with their genotype and gut microbiota to affect health [16, 17].
157 Accordingly, The Manitoba Personalized Lifestyle Research (TMPLR) study will involve the
158 coordinated collection of data related to socio-economic status, geography, nutrition, physical
159 activity, sleep, early life experiences, and health systems usage, in conjunction with the analysis
160 of genetics, gut microbiota, and risk factors for chronic conditions such as obesity, hypertension,
161 T2D, CVD, and CKD. After establishing the baseline characteristics of this study cohort,
162 administrative health records will be used retrospectively to examine the developmental origins
163 of health and disease [18], and prospectively to track and investigate the development of chronic
164 disease in the future, starting at 5 years after the initial study is complete. Consent will be
165 obtained to contact study participants for further clinical assessments, contingent on future
166 funding.

167 Data from this study will provide an ideal opportunity for the exploration and potential discovery
168 of new interactive mechanisms through which lifestyle factors affect health. We will be looking

169 to collaborate with other existing studies [19-21] with overlapping measures to replicate such
170 findings, or increase sample size. Findings from this research may be useful in guiding both
171 clinical and health policy decisions, and will also facilitate the design and testing of personalized
172 health promotion strategies. For example, if we are able to identify interactions between lifestyle
173 factors and disease risk, such as a genetic variant that associates with short sleep to negatively
174 impact health, a follow-up study could be designed looking to improve sleep hygiene specifically
175 in the group with the risk variant.

176 **Methods**

177 **Design**

178 This is an exploratory observational cohort study with retrospective and prospective utilization of
179 secondary data from administrative health records (**Figure 1**). The Strengthening the Reporting
180 of Observational Studies in Epidemiology (STROBE) guidelines were followed where applicable
181 in the development of this protocol manuscript [22].

182 **Setting**

183 Urban (Winnipeg) and rural (Morden, Winkler, Carman, Steinbach) areas with road access in
184 southern Manitoba, Canada.

185 **Objectives of the study**

186 The objective of this study is to explore the complex interactions that exist between lifestyle,
187 genetics, and gut microbiota, and how these relate to risk factors for chronic conditions,
188 especially obesity, hypertension T2D, CVD, and CKD in Manitoba.

189 **Inclusion and exclusion criteria**

190 A sample of 800 Manitobans aged 30-46, stratified by sex, BMI, and geography (**Table 1**) are
191 being recruited. Participants must have lived in Manitoba for a minimum of 5 years. Women
192 who are pregnant or lactating are not eligible to participate. Additionally, because it is expected
193 that very few of the 800 Manitobans who join TMPLR study from the general public will have
194 reduced kidney function (eGFR <30 ml/min), 40 participants from Manitoba (20 female, 20
195 male, with no set stratification based on BMI or geography) who have severely reduced kidney
196 function are being recruited from the renal health clinic at Seven Oaks General Hospital
197 (SOGH), Winnipeg, MB. Therefore, the study has a recruitment goal of 840 participants.

198 **Recruitment**

199 Participants are recruited through the use of printed flyers, online advertisements purchased via
200 Google, Facebook, and Twitter ad platforms and social media accounts, appearances in local TV,
201 radio, and print media, and direct contact with community groups, such as churches, sports
202 leagues, and community clubs. All patients who receive care in the SOGH renal health clinic,
203 who are aged 30-46, have been living in Manitoba for a minimum of the last 5 years, and are
204 able to provide informed consent are approached to enroll in the study as well.

205 **Sample size**

206 The sample size of TMPLR study was selected based on considerations of feasibility of
207 recruitment, costs, and logistics. However, given established values from other sources [23] and
208 our anticipated sample size of 840 participants, we estimate that we will have an 80% power (5%
209 significance, 2-sided) to detect a minimum body fat difference of 2.5% for rare exposures (i.e.,

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3 210 experienced by 10% of participants, such as smoking) and 1.7% for more common exposures
4 211 (experienced by 25% of participants, such meeting the Canadian recommended 150 minutes of
5 212 moderate-to-vigorous physical activity). Additional estimated minimum detectable differences
6 213 are presented in **Table 2**. These lower limits should allow for the detection of clinically
7 214 meaningful changes in these outcomes.
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11 216 **Data Collection and Assessments**

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13 217 On two consecutive days, participants come to either the urban TMPLR study site at the
14 218 Richardson Centre for Functional Foods and Nutraceuticals, University of Manitoba or
15 219 TMPLR's mobile research unit which travels to other areas of Winnipeg and southern Manitoba.
16 220 TMPLR's mobile research unit is a custom built 12-meter mobile lab which is equipped with
17 221 phlebotomy area, a dual-energy X-ray absorptiometer (DXA) and a bicycle ergometer with a
18 222 metabolic cart. During this visit, participants complete questionnaires, undergo various health
19 223 assessments, provide urine and fecal samples, and have fasting blood samples taken (**Figure 2**,
20 224 **Table 3**). The same protocols at were followed at both sites.
21

22 225 ***Questionnaires***

23
24 226 Questionnaires capture socio-demographic characteristics, personal and family medical history,
25 227 smoking (including electronic cigarette use), current diet (three Automated Self-Administered
26 228 24-h (ASA24) Dietary Assessment Tool recalls , Mindful Eating Questionnaire [24], Diet
27 229 History Questionnaire [25] and The Three-Factor Eating Questionnaire [26]), alcohol
28 230 consumption, physical activities, frailty using the Modified Fried Criteria [27], stress, sleep
29 231 (Pittsburgh Sleep Quality Index [28]), cognition (Montreal Cognitive Assessment Questionnaire
30 232 [29]), and childhood retrospective circumstances (adapted from the US Panel Study on Income
31 233 Dynamics [30]).
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34 234 ***Anthropometric assessment***

35 235 Weight is measured after participants change into lightweight scrub tops and bottoms, with shoes
36 236 removed, to the nearest 0.1 kg using a digital calibrated floor scale (7562EF, Taylor Precision
37 237 Products, Oak Brook, IL, USA). Height is measured, without shoes, to the nearest 0.1 cm using a
38 238 stadiometer (Model 206, SECA North America, Chino, CA, USA). BMI is calculated in kg/m^2 .
39 239 Waist circumference is measured in triplicate, to the nearest 0.1 cm at the umbilicus, between the
40 240 last rib and the iliac crest using a fibreglass tape measure. Hip circumference is measured in
41 241 triplicate at the widest portion of the buttocks and hips using a fibreglass tape measure. Body
42 242 composition including fat mass, lean mass, percent body fat, visceral adipose tissue (VAT), and
43 243 bone mineral density (BMD) are assessed using dual-energy X-ray absorptiometry (DXA, Lunar
44 244 Prodigy Advance, GE Healthcare, Mississauga, ON, CAN) [31]. Scans are taken of the whole
45 245 body, femoral neck, L1-L4 of the spine, and the non-dominant forearm.
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49 246 ***Clinical health assessment***

50 247 Participants' systolic and diastolic blood pressures are measured in triplicate, on the non-
51 248 dominant arm in a sitting position using a validated oscillometric blood pressure monitor
52 249 (BP760CAN, Omron, Burlington, ON, CAN). Participants are required to rest for 5-10 minutes
53 250 before taking the measurement. Pulse wave velocity and augmentation index are measured on the
54 251 non-dominant arm in a sitting position using a Mobil-O-Graph PWA Monitor and the HMS
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252 Client Server Software (I.E.M Gmbh, Stolberg, Germany) according to the manufacturer's
253 protocol on two consecutive days [32].

254 *Collection of bio-specimens*

255 Blood, urine, and fecal samples are obtained from study participants (**Supplementary**
256 **protocols**). Fasting blood samples are collected on two consecutive days via venipuncture by
257 trained phlebotomists. Two blood samples on consecutive days are required to undertake the
258 isotopic assessment of fractional cholesterol and triglyceride synthesis rates. Participants are
259 asked to collect two urine samples at home; one sample is obtained prior to going to bed, and a
260 second of the first morning void upon waking up. Participants also collect a fecal sample; they
261 are provided a collection kit and instructed to collect a single sample from 3 separate places on
262 the stool using a spoon attached to the cap of the collection tube. Participants are instructed to
263 store the collected samples fecal in their household -20°C freezer with a provided ice pack, and
264 urine samples in the fridge, until transport back to the study center, using provided ice pack for
265 temperature control, where they are aliquoted and then stored at -80°C for future analysis [33].

266 *Clinical chemistry in blood and urine*

267 Clinical chemistry, including lipid profile, glucose, insulin, and renal and liver profiles will be
268 measured via automated clinical chemistry analyzers (Cobas C111, C311 and e411, Roche
269 Diagnostics Laval, QC). Blood and urine biomarkers such as leptin, glucagon, and melatonin
270 will be measured via ligand binding assay (LBA) or enzyme-linked immunosorbent assay
271 (ELISA). Red blood cell and plasma fatty acids will be measured by gas chromatography with
272 flame ionization detections (GC-FID)[34]. Non-cholesterol sterols will be measured in plasma
273 using GC-FID and mass spectrometry (MS) [35]. Vitamin C concentrations in the blood will be
274 measure by high pressure liquid chromatography (HPLC) [36].

275 *Microbiome analyses in fecal samples*

276 Fecal samples will be subjected to genomic DNA extraction (Zymo Research, CA, USA)
277 following the manufacturer's protocol. Experimental negative controls will be included in
278 extraction protocols to confirm the reliability and consistency of the extracted nucleic acid. The
279 V4 hypervariable region of 16S rRNA gene will be amplified, the sequencing library will be
280 generated as described previously [37] and sequenced at the Gut Microbiome Laboratory,
281 University of Manitoba. Samples will be multiplexed at the rate of 200 per run aiming for an
282 average sequencing depth of 50,000 sequences per sample. The sequencing data will be
283 deposited into the Sequence Read Archive (SRA) of NCBI (<http://www.ncbi.nlm.nih.gov/sra>)
284 and accession numbers will be provided for future access.

285 *Deuterium oxide administration*

286 After the blood sample collection on day one, participants are given 0.7 grams of deuterium
287 oxide per kilogram of estimated body water to drink. Body water is estimated as body weight
288 (kg) x 0.60. This deuterium administration is used to enrich the body's water pool for the
289 assessment of fractional cholesterol and triglyceride synthesis rates [38-40].

290 *Physical activity and capacity testing*

Physical activity level in TMPLR study participants is assessed using accelerometers (Actigraph GTX3bt, Penscola. FL, USA) worn for 1 week [41, 42]. Muscle strength is measured using a hand grip dynamometer. Cardiorespiratory fitness is assessed using a submaximal bike protocol which includes heart rate monitoring, and a metabolic cart (VMAX Encore, Carefusion, Unionville, Ont., Canada) to measure oxygen consumption and CO₂ output. Functional walking ability is assessed using a 5-meter gait speed test. Additionally, depressive symptoms, obesity history, frailty, low physical activity, and cognitive impairment are assessed by validated questionnaires [43-45].

299 ***Sleep assessment***

Sleep in TMPLR study participants is measured objectively using accelerometers (Actigraph GTX3bt, Penscola. FL, USA [46]) worn for a week and subjectively by questionnaire (Pittsburgh Sleep Quality Index [28]). While there is a strong relationship between objective and subjective sleep reports, TMPLR study is collecting both because discrepancies may provide important clinical information reflecting early dysfunction [47, 48].

305 ***Dietary assessment***

Study participants complete the Canadian version of the Diet History Questionnaire (DHQ) [25], which estimates the intake of common food items and includes portion size and dietary supplement questions. This questionnaire is on a TELEform for scanning data entry and creation of the data files. Participants also complete the Mindful Eating Questionnaire [24] to assess awareness of the physical and emotional sensations associated with eating, and The Three-Factor Eating Questionnaire [26] to assess dietary restraint, disinhibition and hunger in relation to eating. Participants also complete three dietary recall surveys using the Automated Self-Administered 24-hour Canada (ASA24®, NCI, Rockville, Maryland U.S. <http://asa24.ca/>) [49] dietary assessment tool, a web-based tool that enables multiple, automatically coded, self-administered 24-hour recalls. Participants enrolled from March 2016 to February 2017 used the ASA24-Canada-2014 edition; those enrolled after February 2017 used the ASA24-Canada-2016 edition. Both ASA24-Canada-2014 and ASA24-Canada-2016 use the same nutrient databases.

318 ***Early life experiences***

Early-life exposures spanning the critical time windows of fetal development, birth, infancy and early childhood are documented in three ways: 1) through linkage with administrative health records (see Linkage to administrative health data), 2) by self-report, and 3) by maternal report. Administrative health data will provide method of birth, gestational age, birth weight, diagnosis codes for post-delivery hospitalization, and post-delivery drug prescriptions. Mothers of TMPLR study participants are asked to complete a TMPLR Mother's Questionnaire, adapted from the Nurses' Health Study [50], capturing key pregnancy, birth, and postpartum events such as method of birth; gestational age and birth weight; socioeconomic status at birth; maternal pre-pregnancy BMI and gestational weight gain; maternal smoking and diabetes during pregnancy; maternal prenatal care; breastfeeding initiation, exclusivity and duration; stressful life events during pregnancy and postpartum; and severe illness requiring hospitalization during infancy or early childhood. Early childhood socioeconomic status [51, 52] and stressful life events [53, 54] are also self-reported by TMPLR participants using the Childhood Retrospective Circumstances Questionnaire, adapted from the US Panel Study of Income Dynamics [30].

333 **Data quality assurance and control**

334 Methods of data collection (questionnaires, anthropometric assessment, and clinical health
335 assessment) were standardized across the urban and mobile TMPLR study sites. Training of
336 TMPLR study staff involved in data collection and data entry is regularly refreshed and all staff
337 handling participant data are trained in compliance with the Manitoba Personal Health
338 Information Act (PHIA). All data will be entered in the secure digital platform. A TMPLR study
339 data model has been created to help in visualizing the different types of data the digital platform
340 will contain. (**Supplementary figure 1**)

341 **Linkage to administrative health data**

342 At enrollment, TMPLR participants are asked to provide their PHIN and grant permission to link
343 their study data with administrative health records (including hospital discharge abstracts,
344 physician billing claims, and prescription records). These data are accessed through the Manitoba
345 Centre for Health Policy (MCHP) Population Research Data Repository [55] and linkage is
346 achieved using the PHIN, following the standard procedures established by the MCHP and the
347 Manitoba Health Information Privacy Committee. The data linkage is used to capture
348 retrospective information on early life as well as prospective information on numerous health
349 outcomes, including diagnosis of hypertension, T2D, CVD, and CKD.

350 **Statistical analyses**

351 Statistical analyses will be undertaken in consultation with biostatisticians from the George and
352 Fay Yee Centre for Healthcare Innovation (CHI) at the University of Manitoba. Lifestyle factors
353 will primarily be used as explanatory variables, with chronic disease biomarkers or disease
354 presence/absence as outcomes, in multivariable regression models. Moderating or mediating
355 effects of genetics, gut microbiome, clinical characteristics, socio-economic status, and
356 environmental factors will be explored. The potential confounding effects of health status and
357 healthcare use on variable relationships will be examined using techniques such as propensity
358 score or instrumental variable models [56-58].

360 Techniques appropriate for high-dimensional data will be adopted where needed. For example,
361 clustering of lifestyle risk factors will be examined using latent variable modeling techniques
362 (i.e., latent class analysis). Dimension reduction techniques for omics data, such as microbiome
363 and genetic markers, will be applied [59].

365 The bioinformatics and statistical analyses of microbiome data will be performed as described
366 previously [37] and will be updated based on recommendations and technology advancements
367 between now and the point of processing of samples. Overall microbiota community structures,
368 alpha diversity metrics, and relative abundances of operational taxonomic units (OTUs), will be
369 tested for associations with lifestyle and health measures, with appropriate adjustment for
370 multiple comparisons.

372 Non-response bias or inability to collect certain data, may affect the validity of analyses for
373 survey data or biological measures, necessitating the use of multiple imputation methods if the
374 pattern of missing data is deemed to be ignorable [60]. For non-ignorable missing data, selection
375 and pattern mixture models will be examined in sensitivity analyses [61]. Due to the use of non-
376 random sampling there is a risk of selection bias; survey weights and weighting of responses
377 may be used to address this bias. Standardization or adjustment techniques may be used to
378 address bio-specimen measurement error bias [62].

379
380 Specialized methodological investigations will be conducted for: 1) psychometric analyses of
381 scales, including testing for differential item functioning and measurement invariance [63-65], 2)
382 development of chronic disease risk prediction models [66, 67], 3) techniques to evaluate the
383 quality of linked databases, including their accuracy, reliability, and completeness [68], 4) robust
384 statistical methods for the analysis of outcome measures with non-normal (e.g. skewed)
385 distributions [69, 70].

386
387 **'Patient and Public Involvement'** Three focus group, one for healthcare providers and two for
388 general public, and a public forum were held in the early design stages of this study to obtain
389 input from Manitobans, on the study design and recruitment strategies. A study advisory board
390 was also formed, and meets on a bi-annual basis. This advisory board includes healthcare
391 providers, health researchers, and members of the public. The board provides input regarding
392 study recruitment, progress and conduct, and will also provide input and suggestions regarding
393 the dissemination of study results.

394 395 **Provision of clinical results to participants**

396 Individual results of the anthropomorphic measurements, blood pressure, pulse wave velocity,
397 augmentation index, body composition, bone density, full lipid profile, fasting blood glucose,
398 and renal and liver profile are to be provided to participants. Participants are referred to their
399 primary care providers for further management if their results are beyond clinical reference
400 ranges. Participants will not be provided their genetic and microbiome information.

401 **Ethics and dissemination**

402 Explicit informed consent is obtained from each individual prior to participation in the study.
403 Eligible participants are verbally informed by trained research personnel regarding the nature and
404 purpose of the study, given time to decide whether or not to participate, and have any questions
405 or concerns answered prior to consent and at any point throughout the study. All participants are
406 informed that they may withdraw from the study at any time without penalty and are
407 remunerated for the portion of the study that they have completed up to that point. The full
408 remuneration for study participation is \$100 Canadian dollars, provided as cash or as a gift card.

409 Ethics approval has been obtained from the University of Manitoba Health Research Ethics
410 Board prior to participant recruitment (protocol# HS18951). The study protocol has also been
411 reviewed and approved by the Manitoba Health Information Privacy Committee in regards to the
412 collection and use of PHIN, The St. Boniface Hospital Research Review Committee in regards to
413 the processing of samples at the hospital, and the Winnipeg Regional Health Authority (WRHA)
414 Research Access and Approval Committee (RAAC), the Southern Health Research Ethics Board,
415 and the Interlake-Eastern Regional Health Authority Regional Ethics Committee, in regards to
416 the study taking place in those health regions.

417 Data analysis, release of results, and publication of initial manuscripts are scheduled for 2019.
418 Findings will be shared in peer-reviewed journals, and at regional, national, and international
419 scientific conferences. Data and findings will also be presented to healthcare policymakers
420 within Manitoba, to develop preventive strategies that reduce chronic conditions with the
421 intention of reducing healthcare costs. Funding applications for future clinical follow in this
422 study population have been submitted starting in 2017.

423 **Discussion**

424 TMPLR study has been uniquely designed to provide cross-sectional, retrospective and
425 prospective observations that will improve our understanding of how lifestyle factors interact
426 with each other and factors such as genetics and the gut microbiome to influence health and the
427 risk of obesity, T2D, CVD, and CKD. The coordinated collection of lifestyle-gene-environment-
428 microbiota-health data, including objective measurements such as DXA, activity monitoring,
429 stable isotopic tracer methodologies, and direct measurement of physiological biomarkers;
430 combined with the ability to retrospectively assess and prospectively follow health outcomes in
431 participants using administrative health records, represents an unprecedented opportunity to
432 collect data which can be used to improve chronic disease prevention and management.

433 Due to the voluntary non-random recruitment of participants, there may be an under-
434 representation of those with lower health awareness, financial means, access, or time to
435 participate. Attempts to counteract this are implicit in the stratified recruitment design.
436 Comparisons between TMPLR study participants and general Manitoban population
437 demographics may allow assessment of potential selection biases. A healthy volunteer effect
438 may impact the ability to detect weak associations between lifestyle and disease risk, but this
439 may attenuate with longer follow-up using administrative health data.

440 Given a projected sample size of 840 participants may be low for some of research questions that
441 will be investigated, therefore harmonization and linking of data across multiple cohorts may be
442 required. We will be looking to other studies which have undertaken overlapping measurements
443 in order to increase sample sizes. The Canadian Longitudinal Study on Aging [19], the Toronto
444 Nutrigenomics and Health [20], and The LifeLines DEEP [21] studies among others will be
445 approached regarding the potential of data harmonization and cross-replication. TMPLR study
446 will also be available to other researchers who are interested in collaboration or using the data for
447 cross-replication.

448 In summary, TMPLR study will provide a unique platform of extensively phenotyped
449 individuals that will be used to explore the interactions between lifestyle factors that associate
450 with the development of, or protection from, obesity, hypertension, T2D, CVD, and CKD. The
451 findings from this research platform will subsequently be used to develop and test preventive and
452 restorative lifestyle and health strategies with the aim of improving the health and reducing
453 healthcare costs at the individual and population levels.

454 **Study status**

455 Data collection started in March 2016. As of the August 15th 2018, data collection is ongoing
456 and has passed 800 participants. Data collection is expected to end in December 2018.

457 **Acknowledgements**

458 The authors would to thank all the Manitobans who have participated in this study, without your
459 valuable contributions we would not be able to undertake this research. The authors would also
460 like to thank the Manitobans who took part in focus groups, and who joined the study advisory
461 board, for their important contributions to this study. Finally, the authors would like to
462 acknowledge the amazing staff involved in making TMPLR study a reality, in particular
463 Stephanie Jew, Sandra Castillo-San Juan, Jeann Buenafe, Meaghan Rempel, Katrina Cachero,
464 Mark Pinder, Eden Vergara and Kamlesh Patel.

465

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3 466 **Author contributions**

4
5 467 DSM and RCM developed the original concept of the study for the original grant application
6 468 with input from co-investigators. DSM prepared the drafts of the study protocol manuscript and
7 469 compiled feedback and changes from other authors. RCM and MG assisted in the preparation of
8 470 the study protocol manuscript. PF developed the branding and logo for TMPLR study, and the
9 471 manuscript figures and tables. NH prepared the data model and was involved in the public
10 472 engagement. SB (project lead, indigenous health), HB (project lead, nutrition), JC, TAD (project
11 473 lead, physical activity), PKE (project lead, genetics), EK (project lead, gut microbiome), LML
12 474 (project lead, biostatistics), DEM (project lead, sleep), SBM, AR, NT, MBA (project lead,
13 475 developmental origins of chronic disease), and PJJ (Director) are study co-investigators, and
14 476 were all involved in writing the original grant application. All authors have carefully read,
15 477 contributed to, and approved the final version of the study protocol manuscript.

16
17
18 478 **Funding statement**

19 479 This work is supported by a grant from Research Manitoba and the Province of Manitoba.
20 480 Financial and in-kind support for the TMPLR program was also provided by the Richardson
21 481 Centre for Functional Foods and Nutraceuticals, the George and Fay Yee Centre for Healthcare
22 482 Innovation, the University of Manitoba Office of Research Services, the University of Manitoba
23 483 Faculty of Agricultural and Food Sciences, and The Wellness Institute and the Chronic Disease
24 484 Innovation Centre at Seven Oaks Hospital. MG is funded by the Frederick Banting and Charles
25 485 Best Canada Graduate Scholarships-Master's. MBA holds a Canada Research Chair in the
26 486 Developmental Origins of Chronic Disease. PJJ holds a Canada Research Chair in Nutrition and
27 487 Functional Foods. These entities had no role in the design of the project.

28
29
30 488 **Competing interests statement**

31
32 489 DSM, RCM, MG, SB, HB, JC, TAD, PKE, PF, NH, EK, LML, DEM, SBM, AR, NT, MBA, and
33 490 PJJ have no competing interests to declare.

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Figure 1. The Manitoba Personalized Lifestyle Research Study overview

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Figure 2. The Manitoba Personalized Lifestyle Research Study participant schedule

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Table 1. The Manitoba Personalize Lifestyle Research Study recruitment targets by strata

Age	30-46 years	
	N=800	
Sex	400 Males	400 Females

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50% Male 50% Female								
Geography 72% Urban 28% Rural	288 Urban males		112 Rural males		288 Urban females		112 Rural females	
BMI 40% Normal (BMI <25) 60% Overweight (BMI ≥ 25)	116 Urban males BMI <25	172 Urban males BMI ≥ 25	45 Rural males BMI <25	67 Rural males BMI ≥ 25	116 Urban females BMI <25	172 Urban females BMI ≥ 25	45 Rural females BMI <25	67 Rural females BMI ≥ 25
+40 participants with severely reduced kidney function (eGFR <30 ml/min), 20 female, 20 male, with no set stratification based on BMI or geography								

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Table 2. The Manitoba Personalized Lifestyle Research (TMPLR) study estimated minimum detectable differences

Variable	Mean or median used	Standard deviation used	Minimum difference at 10% exposure (percentage of mean)	Minimum difference at 25% exposure (percentage of mean)	References
Body fat (%)	41.3% Females 27.8% Males	7.7% 6.6%	2.5% (6.0)	1.7% (4.0)	[23]
Lumbar bone mineral density (BMD; g/cm ²)	1.042 Females 1.058 Males	0.121 0.127	0.041 (3.8)	0.028 (2.6)	[71]
Glomerular filtration rate (GRF; ml/min per 1.73 m ²)	107.6	16.8	5.4 (5.0)	3.8 (3.5)	[72]

Systolic blood pressure (mmHg)	116	12	6.5 (5.6)	4.5 (3.9)	[73]
Fasting Glucose (mmol/L)	4.94	0.61	0.20 (4.0)	0.14 (2.8)	[73]
Fasting insulin (μ IU/mL)	7.83	7.50	2.40 (30)	1.67 (21%)	[74]
LDL cholesterol (mmol/L)	2.79	0.67	0.22 (7.8)	0.15 (5.4)	[73]
Waist circumference (cm)	80	10	3.2 (4)	2.2 (2.75)	[73]

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686 **Table 3. The Manitoba Personalized Lifestyle Research (TMPLR) study data, assessment**687 **tools and biological samples**

<i>Characteristic</i>	<i>Data</i>	<i>Method, Instrument or Source</i>
<i>Sociodemographic</i>	Date of birth, Sex, Ethnicity, Marital status	TMPLR study questionnaire
<i>Medical</i>	Personal medical history, Family medical history, Medication(s), Pregnancy history Cognition	TMPLR study questionnaire, Administrative health records Montreal Cognitive Assessment [29]
<i>Lifestyle</i>	Tobacco/smoking/vaping use, Alcohol use, Unintentional weight loss, Exhaustion, Depression	TMPLR study questionnaire
<i>Physical activity</i>	Frailty	Modified Fried Criteria [27]
	Physical activity	Paffenbarger physical activity index, Actigraphy [41, 42]
	Predicted VO2 max	Modified YMCA bike test with metabolic cart
<i>Nutrition</i>	Dietary patterns and habits	Mindful eating questionnaire[24], three-factor eating questionnaire [26], automated 24-hour dietary recall [49], Canadian dietary history questionnaire [25]
<i>Early life</i>	Childhood health, socio-demographic and socioeconomic status; Parental employment history	Childhood retrospective questionnaire, adapted from the US Panel Study on Income Dynamics[30]

	Maternal: pregnancy events, obstetrical history, infant feeding	TMPLR Mother's retrospective childhood questionnaire, adapted from the Nurses Health Study [50]
<i>Socioeconomic</i>	Employment, Home ownership, Education attainment, Income	TMPLR study questionnaire
<i>Sleep and Stress</i>	Duration of sleep Sleep Quality Perception of stress, Daily life stressors	Actigraphy [46] Pittsburgh sleep quality index [28] Community-based stress and coping survey
<i>Anthropometric</i>	Height Weight Waist circumference, Hip circumference Body fat, Lean mass, bone mineral density	Wall-mounted stadiometer Digital scale Tape measure Dual energy X-ray absorptiometry [31]
<i>Blood pressure</i>	Systolic & diastolic Pulse wave velocity, Augmentation index	Automated sphygmomanometer Mobil-O-Graph oscillometer [32]
<i>Biomarkers</i>	Blood clinical chemistry and biomarker assays Urinary clinical chemistry and biomarker assays Microbiome 16S RNA sequencing	Fasting blood samples Urine samples Fecal sample [37]

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Review only

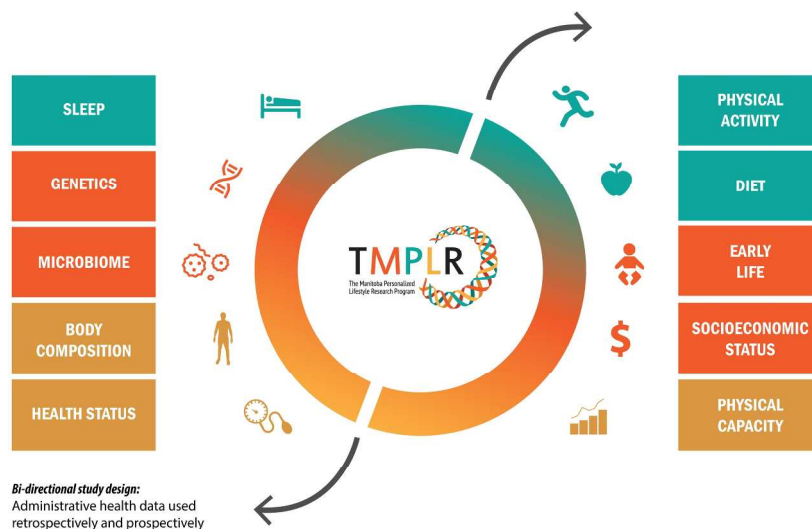


Figure 1. The Manitoba Personalized Lifestyle Research Study overview

230x130mm (300 x 300 DPI)

Review only



PARTICIPANT SCHEDULE

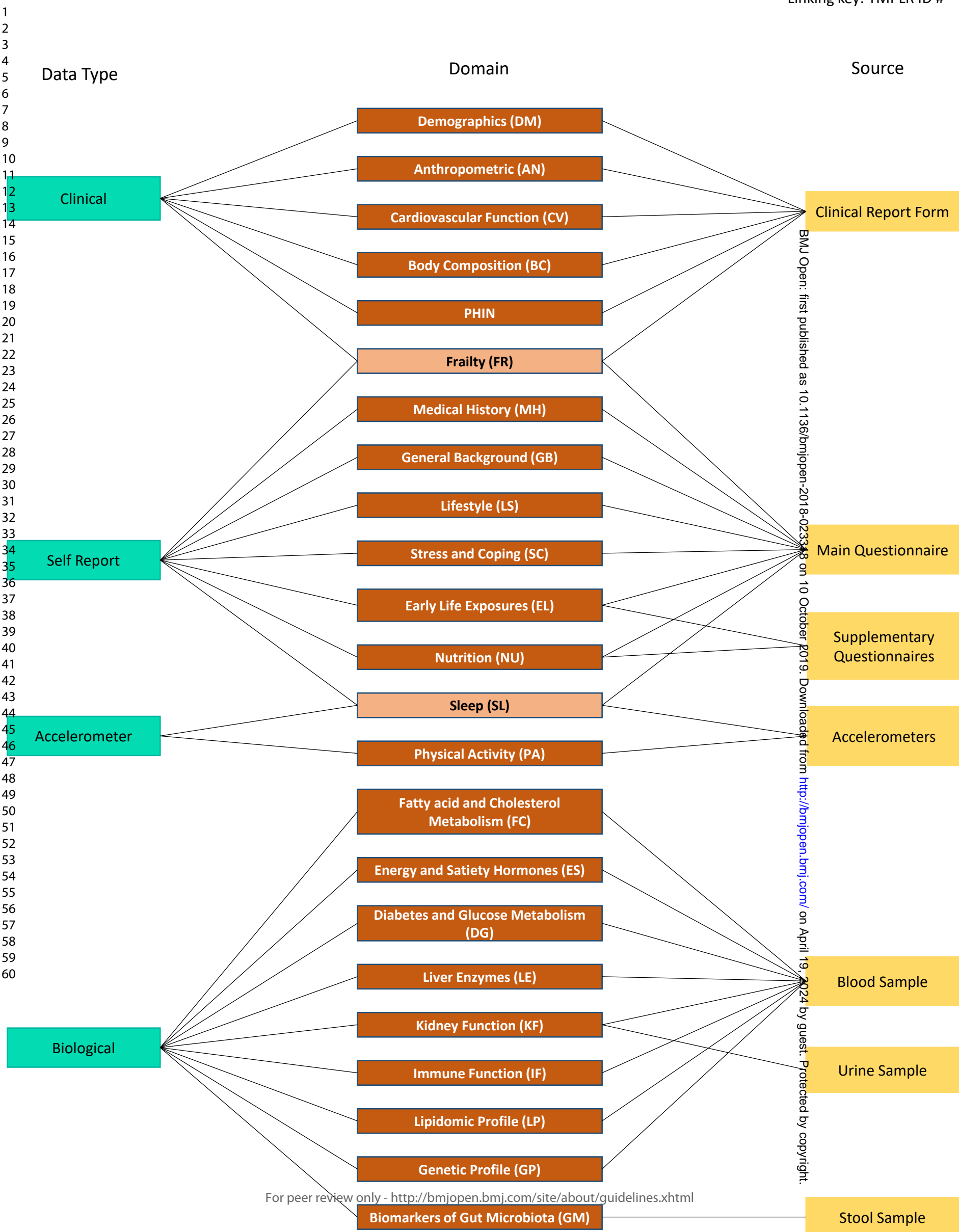
CONSENT PROCESS (completed before Day 1 activities)	
Day 1 (est. 2 hours)	
1	Collect link to administrative health records
2	Anthropometric measurements
3	PWA/PWV & blood pressure
4	Fasting blood samples
5	Oral administration of deuterium
6	Dual energy x-ray absorptiometry (DXA)
7	Fecal & urine sample kits
Day 2 (est. 2 hours)	
1	Fecal & urine collection
2	PWA/PWV & blood pressure
3	Fasting blood samples
4	Physical capacity testing
5	Sub-maximal cardiorespiratory fitness test
6	Start of activity monitoring (return accelerometer after 7 days of tracking)
Take home activities	
1	Questionnaires via website
2	Complete three automated 24-hour dietary recalls
18.02.12-01	

Figure 2. The Manitoba Personalized Lifestyle Research Study participant schedule

279x361mm (300 x 300 DPI)

TMPLR Data Model

Linking key: TMPLR ID #





The Manitoba Personalized Lifestyle Research (TMPLR) Study

Urine Sample Collection Instructions

Please follow these instructions for urine collection. Research personnel will provide you with 2 urine collection cups labeled with time (night and day) and your TMPLR Study ID Number.

- 1. Check your study ID on the collection tubes.** If the study ID is not correct, please correct it yourself, and inform a TMPLR clinical coordinator when you return the samples.
- 2. Collect urine before going to bed tonight in the cup labeled “night”. Please write down the date and time of the sample was collected.** Store the sample in the fridge in the Ziploc bag provided.
- 3. Collect urine from the first time you pee after getting up in the morning, in the cup labeled “day”. Please write down the date and time of the sample was collected.** Store your samples in the fridge in the Ziploc bag provided.
- 4. Please bring the urine samples with you on your day 2 visit.** TMPLR staff will collect the samples from you when you arrive.

If you have any questions, please contact the study coordinator Jeann Buenafe at tmplrtrial@umanitoba.ca or call 204-298-5483.

Thank you for your cooperation!

The Manitoba Personalized Lifestyle Research (TMPLR) Study

Stool Sample Collection Instructions

1. Freeze the ice packs provided by the study once you get home.
2. **Check your study ID** on the collection tubes (the two plastic tubes with blue cap). If the study ID is not correct, please correct it yourself, and inform a TMPLR clinical coordinator when you return the sample.
3. Empty your bladder. Flush toilet. Place the collection unit under the rear part of the toilet seat with the round side pointing towards the back.
4. Have a bowel movement. **Collect 2 samples, one in each plastic tube, from 3 different places of the stool** using the spoon attached to the cap of the collection tubes. **Fill each sample tube about one third of the tube with stool sample.**
5. Close the tube tightly. Place each tube in a Ziploc bag provided. **Write down the date and time of the bowel movement** on the bag. Discard the used collection unit.
6. Wrap the collection tubes with the frozen ice packs, and keep them in the paper bag provided. Keep the collected sample in the freezer.
7. Return the stool samples wrapped with the frozen ice packs on day 2 of the measurements, or as soon as you can. TMPLR staff will collect the sample from you in the paper bag when you arrive.

If you have any questions, feel free to contact the study coordinator Jeann Buenafe at tmplrtrial@umanitoba.ca or call 204-298-5483.



Collection Unit



Collection Tube with Spoon

The Manitoba Personalized Lifestyle Research (TMPLR) Study

Biospecimens collection

Blood samples will undergo analysis for numerous established and emerging health biomarkers, these include: total cholesterol, LDL-C, HDL-C, triglycerides, glucose, AST, ALT, insulin, glucagon-like peptide-1 (GLP-1), leptin, c-reactive protein (CRP), fatty acids, HbA1c, T-regs, serum creatinine, blood urea nitrogen (BUN), non-cholesterol sterols, adipokines, cytokines, vitamin C, fat soluble vitamins, and lipidomic and metabolomics profiling. Gut microbiota analysis will be performed on stool samples. The assessment of gut microbiota is critical as increasing evidence suggests that some of the health effects of physical activity, sleep, and nutrition may be exerted through or modified via the gut microbiota. Participants' DNA will be obtained to determine genetic variations associated with chronic condition risk factors and telomere length measurement.

Urine Collection

Participants will be invited to collect urine from the time subsequent to going to bed (last void at bedtime not collected), to the first morning void. Urine samples will be received on day 2 (see Urine Sample Collection Instructions). Urine samples will undergo analysis for glucose, albumin, creatinine, melatonin, total protein and metabolomics profiling.

Blood collection

Fasting blood samples will be collected on both days (Day 1 and Day 2); they will be identified by participants' ID and separated as indicated (Table 5). Participants should come in fasting state (at least for 12h) and shouldn't take any alcoholic beverage for at least 48h before each visit. A total of 60 mL of blood will be obtained from participants (Appendix 21). Blood will be drawn by a certified phlebotomist and/or a register nurse.

Stool collection

Participants will be asked to collect stool sample from a bowel movement. After this, they will take samples randomly from 3 different places of the stool. Sample will be given to research personnel at the beginning of second appointment. Research personnel will provide instruction to volunteers at the end of the first visit (see Stool Sample Collection Instructions).

The Manitoba Personalized Lifestyle Research (TMPLR) Study

Urine collection processing and collection instructions

Steps	Processing instructions
1	Receive urine sample and store it directly on 4 °C
2	Aliquot tubes should be labeled with participant ID
3	Number of labels required: 7 – 2.0 ml urine labels (if a urine sample was received)
4	If a urine sample is received proceed as follows: <ul style="list-style-type: none"> • Determine the volume of the urine • Pour some urine into a sterile container(to keep) • Aliquot urine into 2 -16 x 100 mm tubes and centrifuge
5	Aliquot as follows: 5 cryovials – 2.0 ml / vial (Seven Oaks) 2 cryovials – 2.0 ml / vial (McMillan)
6	Packaging of samples for transport: These samples must not thaw and must arrive frozen at the research lab Pack a transport box with ice packs and the frozen samples .Place the address label on the box. Ask the courier to return the transport box.



The Manitoba Personalized Lifestyle Research (TMPLR) Study

Blood sample processing and collection instructions

Sample	Blood collection tube	Tube volume	Processing instructions	Aliquoting instructions	Analysis	Date
Serum	Red/grey SST tube	1 x 4mL	<ol style="list-style-type: none"> Invert 5 times Room temp for 30 min Spin for 10 min @ 1000 x g 	<ol style="list-style-type: none"> Aliquot serum into cryovials¹ with brown² caps (0.5mL/tube) Store at -80°C 	Insulin Lipid profile Glucose CRP GLP-1	1, 2
Plasma	CPT tube (sodium heparin)	1 x 8 mL	<ol style="list-style-type: none"> Invert tube 8- 10 times Spin for 30 min @1500- 1800 RCF Resuspend by inverting After addition of PBS spin for 15 min @ 300 RCF Aspirate off as much supernatant without disturbing the pellet Repeat wash in 10mL PBS Resuspend pellet in 3mL freezing medium -10% DMSO (Sigma), 20% FCS (JRH Bioscience) in RPMI1640 (Gibco) 	<ol style="list-style-type: none"> Aliquot entire contents above the gel and transfer to 15 mL Falcon tube Add PBS (w/o Ca++ or Mg++) to make 15 mL Store 1mL aliquots in -70°C using a Cyro-1°C/min freezing container. 	T-Regulatory cells*	1
Plasma heparin	Green top (lithium heparin)	1x 4 mL	<ol style="list-style-type: none"> Invert 8 times Spin immediately for 10 min @1300 x g 	<ol style="list-style-type: none"> Aliquot plasma into cryovials with green³ caps (0.5mL/tube) Store all fractions at -80°C 	C-reactive protein	1, 2
RBC			<ol style="list-style-type: none"> Invert 8 times Spin immediately for 10 min @ 1300 x g 	<ol style="list-style-type: none"> Aliquot RBC into cryovials with red⁵ caps (0.5mL/tube) Store all fractions at -80°C 	Fatty Acid Analysis	1, 2
White blood cells Heparin			<ol style="list-style-type: none"> Invert 8 times Spin immediately for 10 min @ 1300 x g 	<ol style="list-style-type: none"> Aliquot WBC (buffy coat) in 1 (one) Cryo.s™ (RNase and DNase free 	DNA extraction/ Telomere length	1, 2

The Manitoba Personalized Lifestyle Research (TMPLR) Study

Plasma EDTA	Purple top (K2 EDTA)	1X 10 mL	1. Invert 8 times Spin immediately for 10 min @ 1300 x g 2. After addition of Methanol/ EDTA, spin @ 16,000g for 10 min. @ 1300 x g	1. Aliquot plasma into cryovials with yellow caps (1.0 mL/tube) ⁵ 2. Add to 1 plasma aliquot (0.5ML), 1 volume of sample to 4 volumes of 90% methanol/water/1 mM EDTA 3. Place on dry ice for 5 min 4. Store all fractions at -80°C	Ascorbic acid	1,
Plasma EDTA			1. Invert 8 times 2. Spin immediately for 10 min @ 1300 x g	1. Aliquot plasma in cryovials with purple caps (0.5ml/tube) ⁶ 2. Store all fractions at -80°C	Leptin Glucagon Oxidized phospholipids and oxylipins	1,2
Plasma EDTA			1. Invert 8 times 2. Spin immediately for 10 min @ 1300 x g	1. Aliquot RBC into cryovials with purple caps (0.5mL/tube) ⁵ 2. Store all fractions at -80°C	Non-cholesterol sterols	1,2
White blood cells EDTA			1. Invert 8 times 2. Spin immediately for 10 min @ 1300 x g	Aliquot WBC (buffy coat) in 1 (one) Cryo.s™ (RNase and DNase free vials) ⁴ 2. Store at -80°C	DNA extraction/ Telomere length	1,2